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The Mechanism of Sulfur Dioxide Initiated Bronchoconstriction

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THE MECHANISM OF SULFUR DIOXIDE INITIATED BRONCHOCONSTRICTION

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16. ABSTRACT

In vitro studies with unsensitized guinea pig lung fragments (ULF) incubated with 10 to 200 mM concentrations of ammonium ion demonstrated the release of substantial quantities of histamine. Of the anions tested, sulfate was the most potent, while nitrate and acetate ions were of intermediate potency and chloride less potent. Absorption of sulfate ion from the airways of the isolated ventilated and perfused rat lung (IVPL) appears to be by simple diffusion and to be enhanced in the presence of ammonium ions at 0.01 μmole/lung. Manganous ion was an exception and showed no enhancement. The $t_{\frac{1}{2}}$ for the initial rate of sulfate absorption was 8.4 ± 1.8 minutes. The administration of 1 μmole $(NH_4)_2SO_4$ intratracheally led to a rapid decrease in the respiratory volume of the lung, an effect which could be blocked by prior perfusion with mepyramine maleate (10^{-5} M). Experiments in vivo demonstrate that sulfate ion removal from the rat lung airways appears to be simple diffusion with $t_{\frac{1}{2}}$ of 34.5 minutes. Deviations from physiological pH of the sulfate containing medium and the addition of certain cations (0.1 nanomole/lung) enhance sulfate absorption. In all systems tested, there is a positive correlation between the irritant potential associated with a specific sulfate salt aerosol and the rate at which sulfate ions present in such solutions are removed from the lung.

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FORWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Atmospheric sulfur oxides exist in chemically complex particulates of the respirable size range. Inhalation of these particulates represents a potential health hazard. Before one can estimate the extent of the long-term health hazard to man or espouse a particular strategy for abatement, a clear understanding of the mechanism(s) by which sulfates interact with the mammalian lung is needed. This report provides information concerning the uptake of sulfate salts by the lung, the interaction of sulfate salts with specific hormonal systems in the lung and the potential interrelations between sulfate and heavy metal aerosols as they might exist in the environment. The data reported here describe the uptake and elimination kinetics of sulfate ion in mammalian lungs. The release of histamine by sulfate salts is demonstrated as a potential mechanism of action and as a means by which the varying potency of different chemical salts of sulfuric acid may be explained. These studies illustrate that sulfate aerosols cannot be considered independent of the other inorganic compounds found in respirable particles. The demonstrated removal of sulfate ions from the lung clearly shows that such mechanisms are extant and adds to our knowledge of the pathophysiology of the lung, as it is related to disease processes.

JHKnelson

John H. Knelson, M.D.
Director,
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PREFACE

The release of sulfur oxides into the atmosphere is a most pressing problem. The use of alternative fossil fuel sources other than petroleum will undoubtedly lead to the emission of larger amounts of sulfur into the atmosphere. Atmospheric sulfur oxides exist in chemically complex particulates of the respirable size range. Inhalation of these particulates represents a potential health hazard. Before one can estimate the extent of the long-term health hazard to man or espouse a particular strategy for abatement, a clear understanding of the mechanism(s) by which sulfates interact with the mammalian lung is needed. This report provides the results of a series of experiments into the uptake of sulfate salts by the lung, the interaction of sulfate salts with specific hormonal systems in the lung and the potential interrelations between sulfate and heavy metal aerosols as they might exist in the environment. The data reported here describe for the first time the uptake and elimination kinetics of sulfate ion in mammalian lungs. The release of histamine by sulfate salts is demonstrated as a potential mechanism of action and as a means by which the varying potency of different chemical salts of sulfuric acid may be explained. In sum, these studies illustrate that sulfate aerosols can not be considered independent of the other inorganic compounds found in respirable particles. Hopefully, these data will also provide impetus for detailed studies of electrolyte transport in the lung and its potential relation to disease in man. The demonstrated removal of sulfate ions from the lung clearly shows that such mechanisms are extant and adds to our knowledge of the pathophysiology of the lung, as it is related to disease processes.

Daniel B. Menzel
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ABSTRACT

In vitro studies with unsensitized guinea pig lung fragments (ULF) incubated with 10 to 200 mM concentrations of ammonium ion demonstrated the release of substantial quantities of histamine. Of the anions tested, sulfate was the most potent, while nitrate and acetate ions were of intermediate potency and chloride less potent. An osmotic effect is unlikely since equal concentrations of NaCl failed to release histamine or LDH, a cytoplasmic enzyme into the incubation medium. Drugs known to modulate the anaphylactic release of histamine through the cAMP and cGMP systems had no effect on the ammonium ion mediated release of histamine.

Studies in sensitized guinea pig lung fragments (SLF) demonstrated the known phenomenon of the ability of the cAMP and cGMP systems to modulate antigen-antibody release of histamine. Acetylcholine stimulated the release of histamine while epinephrine and isoproterenol depressed histamine release in accordance with previous reports. Dibutyryl cAMP and phenylephrine failed to have significant effects.

The mechanism of absorption of sulfate ions was investigated. The intracellular sulfate ion space in ULF decreased in the

presence of 50 mM and 100 mM $(NH_4)_2SO_4$ as compared to the presence of Na_2SO_4. Since histamine release occurred only in the presence of $(NH_4)_2SO_4$, the decrease in the intracellular sulfate ion space is probably associated with the degranulation process. The intracellular sulfate space in SLF was significantly decreased also in the presence of 100 mM $(NH_4)_2SO_4$.

In both ULF and SLF, drugs capable of modulating the cAMP and cGMP systems failed to alter the sulfate ion uptake. Sulfate ion absorption does not appear to be highly dependent on metabolic energy. At high concentrations of potent metabolic inhibitors only partial inhibition of sulfate ion uptake was observed. Phloretin has been reported to inhibit chloride and sulfate uptake by human red blood cells, however, phloretin had no effect on the sulfate ion uptake by the lung fragments. The apparent energy of activation for the initial rate of sulfate absorption was found to be 3.1 Kcal for ULF and 3.2 Kcal for SLF.

Using the binding of Acridine Orange (AO) to heparin as a model of histamine binding, the association of AO with heparin was found dependent on the ionic strength of the incubation medium. The total number of binding sites for AO per disaccharide unit was unchanged with increasing ionic strength of medium. Histamine release by inorganic salts may be a simple ionic exchange phenomenon.

The kinetics of sulfate absorption from the airways of the isolated ventilated and perfused rat lung (IVPL) are presented. Absorption of sulfate ion appears to be by simple diffusion and to be enhanced in the presence of ammonium ions at 0.01 μmole/lung. Manganous ion was an exception and showed no enhancement. The $t_{\frac{1}{2}}$ for the initial rate of sulfate absorption was 8.4 ± 1.8 minutes. Sulfate ions introduced into the vasculature have the same volume of distribution and mean transit time within the lung as blue dextran, a compound unlikely to leave the intravascular space. Thus, sulfate ion absorption in the rat IVPL is unidirectional. The administration of 1 μmole $(NH_4)_2SO_4$ intratracheally led to a rapid decrease in the respiratory volume of the lung, an effect which could be blocked by prior perfusion with mepyramine maleate (10^{-5} M). Ammonium sulfate caused a rapid release of a large portion of the histamine stores into the lung perfusate. Neither LDH nor prostaglandins were released by any ions tested.

Experiments _in vivo_ demonstrate that sulfate ion removal from the rat lung airways appears to be simple diffusion with $t_{\frac{1}{2}}$ of 34.5 minutes. Deviations from physiological pH of the sulfate containing medium and the addition of certain cations (0.1 nanomole/lung) enhance sulfate absorption. As in the case of the IVPL, manganous ion failed to stimulate absorption.

In all systems tested, there is a positive correlation between the irritant potential associated with a specific sulfate salt aerosol and the rate at which sulfate ions present in such solutions are removed from the lung. From these data a model is proposed for the absorption of sulfate ions and the release of histamine by ammonium sulfate.

CONTENTS

Foreword iii

Preface iv

Abstract vi

List of Figures x

List of Tables xii

Acknowledgements xiv

1. Introduction 1
2. Conclusions 3
3. Recommendations 5
4. Sulfate Ion Uptake and Histamine Release in Guinea Pig Lung Fragments 7
5. Absorption of Sulfate Ion in the Isolated, Ventilated and Perfused Rat Lung 46
6. Sulfate Ion Absorption in the Rat Lung *In Vivo* . . . 72
7. Discussion 90

References 99

Bibliography 107

LIST OF FIGURES

2.1 Standard Histamine Curve 14

2.2 Standard DNA Curve 18

2.3 Release of Histamine from Lung Fragments by Salts . . . 21

2.4 Dose Response Release of Histamine by Ovalbumin in Sensitized Lung Fragments 27

2.5 Time Course of ^{35}S-Sulfate Ion Uptake by Unsensitized Guinea Pig Lung Fragments 28

2.6 Time Course of ^{35}S-Sulfate Ion Uptake by Sensitized Guinea Pig Lung Fragments 33

2.7 Metachromatic Titration of Acridine Orange with Heparin 37

2.8 Absorption Spectra of Acridine Orange-Heparin Solution in the Presence and Absence of Various Salts 38

2.9 Metachromatic Titration of Acridine Orange by Heparin and Varying Ionic Strengths 39

3.1 Schematic Diagram of the Isolated, Ventilated and Perfused Lung Preparation 51

3.2 Absence of Significant LDH Release into the Effluent of the Perfused Lung 58

3.3 Removal of ^{35}S-Sulfate Ions from the Rat IVPL 59

3.4 Absence of Pulmonary Vascular Uptake of ^{35}S-Sulfate Ions 62

3.5 Release of Histamine into the Lung Effluent by Ammonium Sulfate 65

3.6 Effects of Intratracheal Injection of Salts on Respiratory Volume 67

3.7 Absorption of ^3H_2O in the Rat IVPL 69

4.1 Percent Sulfate Ion Unabsorbed by the Rat Lung *In Vivo* 80

4.2 Percent Sulfate Ion Unabsorbed by the Rat Lung Under Varying pH Conditions 85

5.1 Proposed Mechanism for Sulfate Ion Absorption and Release of Histamine in the Presence of Ammonium Sulfate 97

LIST OF TABLES

2.1 Release of Histamine from Lung Fragments in the Presence of 100 mM Salt Solutions 22

2.2 The Absence of Isoproterenol Mediation on Histamine Release by Sulfate Salts 24

2.3 The Absence of Acetylcholine Mediation on Histamine Release by Sulfate Salts 25

2.4 The Absence of Dibutyryl cAMP Mediation on Histamine Release by Sulfate Salts 26

2.5 Sulfate Ion Uptake in Unsensitized Guinea Pig Lung Fragments 30

2.6 Effect of Metabolic Inhibitors on the Uptake of Sulfate Ion by Guinea Pig Lung Slices 31

2.7 Failure of Pharmacological Agents to Modulate the Uptake of Sulfate Ion by Guinea Pig Lung Slices 32

2.8 Sulfate Ion Uptake in Sensitized Guinea Pig Lung Fragments 34

2.9 Effects of Pharmacological Agents on the Anaphylactic Release of Histamine and Sulfate Ion Uptake in Sensitized Guinea Pig Lung Fragments 36

2.10 Absence of an Effect of Ionic Strength on the Number of Acridine Orange Binding Sites on the Heparin Macromolecule 40

2.11 Release of LDH from Unsensitized Lung Fragments . . . 42

3.1 Distribution of Intratracheal Injection of 0.1 ml Isotonic Sucrose Solution 57

3.2 Effect of Ammonium Ion on the Kinetics of Sulfate Ion Absorption from the Rat IVPL 60

3.3 Effect of Heavy Metal Counter Cation on the Kinetics of Sulfate Ion Absorption from the Rat IVPL 63

4.1 Absorption of ^{35}S-Sulfate in the Rat Lung *In Vivo* . . . 82

4.2 Effect of Counter Cation on Pulmonary Absorption of ^{35}S-Sulfate Ions 83

4.3 Effect of Nickel Chloride Aerosol on Pulmonary Absorption of Sulfate Ion 87

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SECTION 1

INTRODUCTION

According to data obtained by the United States National Air Sampling Network (2) the level of particulate sulfate compounds in urban areas was 10.1 μg/m^3 and in rural areas was 5.3 μg/m^3 in 1967. In the mid 1970's the automobile catalytic converter has become a new source of atmospheric sulfate compounds. It is capable of oxidizing up to 80 percent of the sulfur in the fuels to sulfuric acid.

Environmental exposure to SO_2 and associated particulates has been associated with impairment of pulmonary function (67), as well as increased prevalence of chronic bronchitis (16,69) and increased incidence of acute respiratory disease (30,34). Severe bronchoconstriction due to inhalation of certain sulfate aerosols has been demonstrated by Amdur (5) and Amdur and Corn (6).

The respiratory tract epithelium behaves as a highly porous membrane absorbing a number of solutes (13,14,22). Absorption rates of a wide variety of inorganic and organic solutes have been studied by intratracheal instillation (23,73). The absorption of most compounds investigated follows first order kinetics and appears to be by simple diffusion.

In light of the above considerations, the present study was undertaken to elucidate the mechanism of the observed bronchoconstriction due to the inhalation of certain sulfate salts. The mechanism whereby sulfate ions are removed from the mammalian lung was also investigated.

SECTION 2

CONCLUSIONS

We have been able to demonstrate that unsensitized guinea pig lung fragments (ULF) incubated with a variety of ammonium salts release significant quantities of histamine. The most efficacious, ammonium sulfate (100 mM), shows maximal histamine release after 30 minutes. The ammonium sulfate mediated release is equal to 97% of the total histamine stores. Equal concentrations of sodium sulfate also fail to release histamine, supporting the concept that only certain sulfate salts have biological actions.

The removal of sulfate ions from the airway appears to be predominantly by simple diffusion. Absorption of sulfate ions in the reverse direction, specifically from the vasculature into the lung, could not be demonstrated. At very low doses ammonium ion increases the removal process. The $t_{\frac{1}{2}}$ at doses of 0.05 μmole or greater was 8.4 ± 1.8 minutes. The heavy metal cations tested significantly enhanced the absorption of sulfate ions from the airways. An exception to this rule was manganous ion, which had no effect over control.

In all systems tested there is a positive correlation between the irritant potential associated with a specific sulfate salt (5,9), and the rate at which sulfate ions are cleared from the lung.

Data presented here suggest a correlation between the rate of sulfate ion absorption from the mammalian lung and the reported bronchoconstriction in the presence of certain sulfate salts. Clearly, the rate of sulfate ion absorption is influenced by the cationic species present and the pH of the surrounding extracellular environment. The role of histamine release as a mechanism for the bronchoconstriction action of ammonium sulfate aerosols is strengthened by our data. More research is needed to investigate the possible release of other vasoactive substances by heavy metal sulfate salts.

SECTION 3

RECOMMENDATIONS

Atmospheric sulfate particulates exist in a complex chemical form associated with a large number of heavy metal ions. The exact composition undoubtedly varies with the geographic location and proximity to specific sources of sulfur emission. Nonetheless, the data presented in this report, as well as in the literature, indicate a strong dependence of sulfate ion removal and bronchoconstrictive effect of sulfate aerosols depending on the cation associated with sulfuric acid. A further investigation of the effects of cations (e.g. Zn, Ni, Cd, Co, Hg, Fe, and Mn) released through energy production is needed. A reciprocal relationship may exist whereby sulfate salts may stimulate directly or indirectly the uptake of these ions. Studies of the uptake of these heavy metal ions themselves as well as sulfate ion from the pulmonary lumen are recommended.

The morphological complexity of the lung places inherent limits on the kinetic and mechanistic measurements that can be made with intact animals or perfused, isolated lungs. The development of a model system of isolated lung cells, preferably of sustained and uniform composition in culture, to study the uptake of sulfate and other environmentally present ions is recommended.

Such studies should be applied to generalized principles to overcome the limitations of studies restricted to specific kinds of pollutants. Generalization will allow the application of such data to a wide variety of sources of emission.

The use of pharmacological methods to mimic human disease states, such as bronchitis and asthma, is also recommended. The health hazard of particulate sulfates to specific populations may be greater due to the independent release of histamine by sulfate salts which may not be prevented by natural defense mechanisms.

SECTION 4

SULFATE ION UPTAKE AND HISTAMINE RELEASE IN GUINEA PIG LUNG FRAGMENTS

INTRODUCTION

Amdur (5) and Amdur and Corn (6) measured an increase in pulmonary resistance following the inhalation of zinc ammonium sulfate, zinc sulfate, and ammonium sulfate aerosols. Although ammonium sulfate was the least potent salt, it was many times more irritating than sulfur dioxide (SO_2). These observations raised the question of whether sulfate aerosols arising from SO_2 emitted into the atmosphere were more potent than the parent compound, SO_2. The experiments of McJilton _et al_. (52) support the concept that certain sulfate salts are bronchoconstrictors. Nadel _et al_. (53) have shown that the inhalation of a zinc ammonium sulfate aerosol increases pulmonary resistance in guinea pigs as does inhalation of a histamine aerosol.

The bronchoconstriction, in the presence of certain sulfate aerosols, suggests the possible release of a vasoactive hormone. Histamine is present in significant quantities in guinea pig (19-35 μg/g tissue) lung tissue (66). Drazen and Austen (20) studied the effect of intravenous administration of slow reacting substance of anaphylaxis (SRS-A) which led to a 50% decrease in pulmonary compliance, but had little effect on airway resistance. Bradykinin and histamine had similar effects on compliance but increased airway resistance by 60-140%. These observations led to the investigation of the direct effects of ammonium sulfate and other salts on histamine release from guinea pig lung *in vitro* (17).

Two theories have been proposed for the site of the histamine binding in mast cell granules. Lagunoff *et.al*. (47) have proposed that the negatively charged heparin macromolecule, a major constituent of the mast cell granule, is the binding site for histamine. Alternatively the mast cell granule protein has been proposed by Uvnas *et.al*. (80) as the histamine binding component. Lagunoff (46) has shown that 2.0-2.5 of the 3.0-3.5 anionic sites per disaccharide unit of heparin in rat mast cells are available for binding of histamine *in situ*. Utilizing the metachromasia associated with Acridine Orange binding to heparin to study the ionic interactions between protein, heparin, and histamine, Lagunoff has proposed that heparin *O*-sulfate groups interact with the amine groups of the granule protein, leaving the glucosamine *N*-sulfate groups, some *O*-sulfate groups and the uronic carboxyl groups of the heparin macromolecule to interact with histamine. Stone and Bradley (72) and Lagunoff (46) suggest that the binding sites for Acridine Orange and histamine on the heparin macromolecule are identical.

To date, a process for the uptake and elimination of inspired sulfate salt particulates by the mammalian lung has not been reported. Gunn (36) has proposed a protonatable carrier for both mono- and divalent anion transport in human red blood cells. In the model, the carriers are confined to the membrane of the cell. The carrier (C) can interact with from one to three protons to form species denoted as C_1, C_2 and C_3. C_1 can complex with monovalent inorganic anions such as chloride and bicarbonate. C_2 can complex with divalent inorganic anions such as sulfate. Once complexed, the carrier can transverse the membrane and exchange its complexed anion for one intracellular bicarbonate ion to maintain intracellular electrical neutrality. C_2 can traverse the membrane with one sulfate ion and exchange the sulfate ion for two bicarbonate ions to maintain electrical neutrality.

Gunn et.al. (38) have shown that chloride and sulfate (37) transport in human red blood cells fit this model. Recently Levinson and Villereal (48,49) have demonstrated that sulfate transport in Ehrlich Ascites tumor cells was also consistent with the idea of a carrier mediated or facilitated transport system.

Two animal model systems have been employed extensively to represent the two distinct populations of humans exposed to atmospheric pollution. Normal guinea pigs mimic the majority of the population with unimpaired respiratory functions. Guinea pigs that have been actively sensitized to an antigen, such as ovalbumin, represent a model of atopic or hypersensitive individuals such as asthmatics.

Actively sensitized guinea pig lung fragments release histamine and SRS-A on stimulation by the immunoglobulin E (IgE) mediated antibody-antigen reaction (71). From studies by Orange _et.al_. (57) with drugs known to influence the cellular levels of adenosine 3'5'-cyclic monophosphate (cAMP), it appears that the cAMP system may be capable of modulating the immunological release of histamine and SRS-A from human lung fragments. The β-adrenergic agonists, isoproterenol and epinephrine, which are known to increase intracellular levels of cAMP, inhibited the antigen induced release of histamine and SRS-A from human lung (58), as well as guinea pig lung fragments (65). Agonists have predominantly α-adrenergic action, such as phenylephrine, probably decrease cellular levels of cAMP and enhance the anaphylactic release of histamine and SRS-A (44). Acetylcholine, working through the guanidine 3',5'-cyclic monophosphate (cGMP) system has also been shown by Kaliner _et.al_. (44) to increase the release of these vasoactive substances.

In this chapter, the possible release of histamine by ammonium sulfate and other salts from guinea pig lung fragments are discussed, as well as studies designed to elucidate parameters of sulfate uptake by lung tissue from the two previously mentioned animal model systems. Possible pharmacological modulation of sulfate salt mediated histamine release and the sulfate ion uptake process were also investigated.

METHODS

Active Sensitization of Guinea Pigs: Sensitization was accomplished by the method of Sorenby (70), whereby male Hartley guinea pigs were given a single intraperitoneal injection of 20 mg ovalbumin suspended in 0.5 mg Freund's complete adjuvant. From 3-5 weeks later, identification of sensitized animals was accomplished by placing 2 drops of antigen solution (1 mg/ml in 0.9% NaCl) into one eye of the treated animals. Ten minutes later the intensity of antigen-induced swelling was judged. Swelling of the orbital connective tissue sufficient to lift the rims of both eyelids from the surface of the eyeball was required before the animal was employed in the preparation of the lung fragments. This technique has been used successfully by Taylor and Roitt (76) as a measure of active sensitization of guinea pigs.

Preparation of Lung Fragments: Normal or unsensitized guinea pigs, weighing between 300-400 g were anesthetized with sodium pentobarbital (20 mg/kg) and the lungs excised. The lungs were cut into fragments (75-150 mg) with a razor blade and washed repeatedly with Tyrode's solution until the fragments were free of blood. Randomized lung fragments, weighing 150-200 mg were employed in each incubation flask. All incubations were performed in 3.0 ml of Tyrode's solution. The Tyrode's solution contained 0.9 g NaCl, 0.02 g KCl, 0.02 g $CaCl_2$, 0.01 g $MgCl_2$, 0.1 g glucose, 0.1 g $NaHCO_3$ and 0.005 g NaH_2PO_4 per 100 ml adjusted to pH 7.40.

Anaphylactic Release of Histamine by Ovalbumin: Sensitized lung fragments (SLF) were suspended in 3.0 ml of Tyrode's solution (pH 7.4) containing 0, 2.5, 5.0, 7.5 and 10.0 mg of ovalbumin. Incubations were carried out for 30 minutes at 37°C, the fragments removed and the supernatant assayed for histamine. The total histamine content in SLF was determined by boiling fresh tissue for 8 minutes and assaying the supernatant.

Release of Histamine by Ammonium Sulfate and Other Salts: Unsensitized lung fragments (ULF) were incubated in the presence of varying concentrations (10-200 mM) of the salts under study for 30 minutes at 37°C, the fragments removed, and the supernatant assayed for histamine. The salts studied in this manner were sodium chloride, sodium sulfate, ammonium chloride, ammonium sulfate, ammonium nitrate, and ammonium acetate. Total histamine (μg/g lung wet weight) was determined by boiling fresh ULF for 8 minutes and assaying the supernatant.

Histamine Assay: Histamine was measured spectrophotofluorometrically (66). Two-tenths milliliter of 70% perchloric acid was added to 2.0 ml of sample solution and the mixture incubated for 30 minutes. One milliliter of the supernatant was added to 0.75 g NaCl and then 1.5 ml n-butanol and 0.3 mg 5 N NaOH were added and mixed. The lower aqueous phase was removed by suction and 1.5 ml 1 N NaOH saturated with NaCl was added to the remaining phase and mixed. One milliliter of the upper butanol layer was transferred to 1.5 ml hexane and 1.25 ml 0.1 N HCl and the mixture agitated. The upper organic phase was removed by suction of 0.45 ml of the acid phase and added to 0.1 N NaOH and 0.05 ml of 0.5% methanolic solution of o-phthaldialdehyde. The reaction was stopped after 4 minutes by the addition of 0.05 ml 3 N HCl. After the addition of 1.5 ml distilled water, the fluorescence was measured at 450 nm by excitation at 360 nm in a Turner model 110 Fluorometer(G. K. Turner Associates, Palo Alto, Cal.). A typical standard histamine curve is shown in Fig. 2.1.

Lung Fragment Uptake of ^{35}S-Sulfate Ions: ULF were incubated in the presence of concentrations of 10-100 mM of either sodium sulfate or ammonium sulfate. The fragments were preincubated in these solutions for 10 minutes followed by the addition of ^{35}S-sodium sulfate (1μCi of a 947 mCi/mmole solution) to each incubation mixture. The fragments were incubated in a shaking water bath at 0, 22, or 37°C. Incubations were stopped at times between 0 and 60 minutes, the fragments removed, washed twice in ice cold medium and used in the determination of ^{35}S radioactivity. A plot was made of the natural log of percent uptake versus time, to calculate the rate constants. These values were used in an Arrhenius plot to determine the energy of activation of the uptake process.

The SLF were studied in a similar fashion in the absence and presence of 5.0 mg ovalbumin in the initial incubation medium.

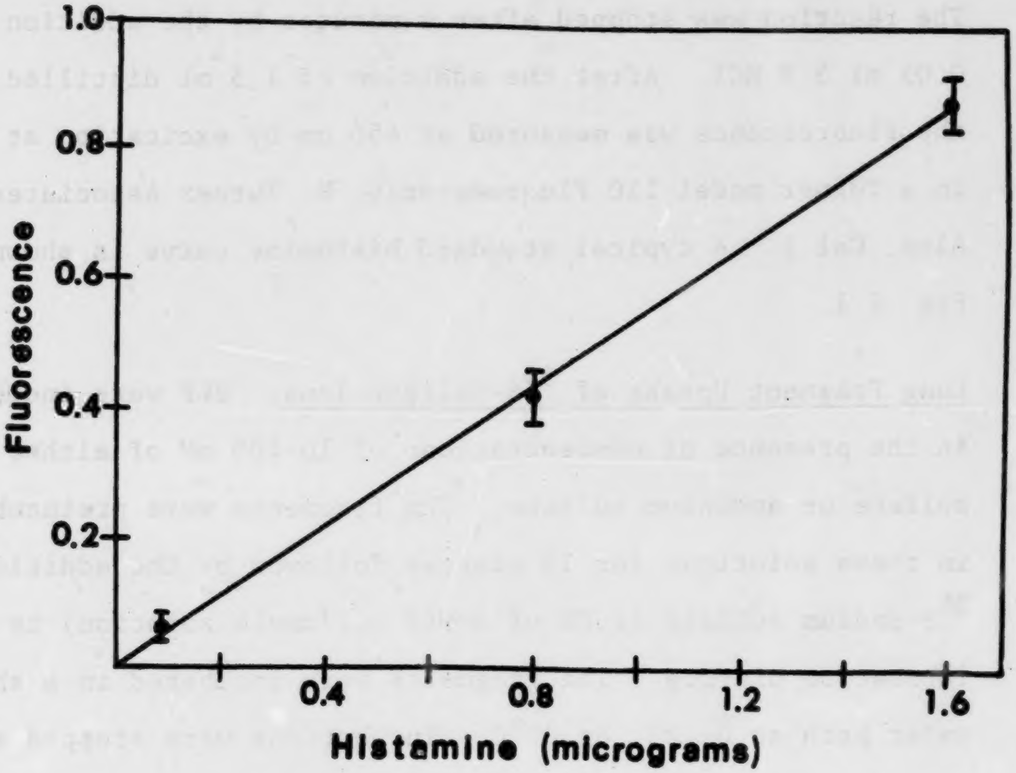


Fig. 2.1. A typical standard histamine curve using histamine diphosphate as a standard. Each point is the mean ± s.e. of 4 determinations.

Pharmacological Modulation of Sulfate Uptake and Histamine Release: ULF were preincubated at 37°C in 3.0 ml Tyrode's solution containing 10^{-3} M sodium cyanide, sodium fluoride, or 2-deoxyglucose, or 10^{-5} M phenylephrine, epinephrine, isoproterenol, dibutyryl cAMP, or phloretin. ^{35}S-sodium sulfate (1 μCi of 947 mCi/mmole solution) was added to each incubation mixture after 10 minutes. Incubations were stopped 30 minutes later, the fragments removed, washed twice in ice cold medium and used in the determination of ^{35}S radioactivity.

Possible pharmacological modulation of salt mediated histamine liberation in ULF was investigated by pre-incubation for 10 minutes with either isoproterenol (10^{-3} M), acetylcholine (10^{-4} M), or dibutyryl cAMP (10^{-6} M). The release of histamine was determined 30 minutes later upon the addition of ammonium sulfate, ammonium chloride or sodium sulfate.

Pharmacological modulation of the sulfate release and IgE-mediated release of histamine in SLF was sought for simultaneously. SLF were pre-incubated in the presence of 10^{-3} sodium cyanide, sodium fluoride, or 2-deoxyglucose or 10^{-5} M phenylephrine, epinephrine, isoproterenol, dibutyryl cAMP, or phloretin. Five milligrams ovalbumin was added to each incubation mixture after 10 minutes for an additional 10 minutes pre-incubation. ^{35}S-sodium sulfate (1 μCi of a 947 mCi/mmole solution) was added to each flask at the end of the second pre-incubation. Incubations were stopped 30 minutes later, the fragments removed, washed twice in ice cold medium and used in the determination of ^{35}S radioactivity. The supernatant of the incubation mixture was used to assay for histamine release.

Determination of ^{35}S Radioactivity: The lung fragments were prepared for liquid scintillation counting by flask oxygen combustion as described by Buyske et.al. (15) and Abou-Donia et.al. (1). The tissue was placed on ashless black filter paper and 0.2 ml of a 10% sucrose solution was added to aid combustion. The samples were dried overnight. The paper containing the dried sample was placed in a platinum basket suspended from a glass hook in a stoppered one liter Erlenmeyer flask containing 5.0 ml deionized water. The flasks were flushed with oxygen for 20 seconds and ignited with a Thomas-Ogg IR igniter (A. H. Thomas Company, Philadelphia, Pennsylvania). A 1 ml aliquot of the resulting solution was added to 10 ml scintillation solution and counted in a Beckman LS-100C liquid scintillation counter. The scintillation medium was a mixture of toluene-Triton X-100 (2:1 v/v) containing 2.79 g/1 2,5-diphenyloxazole and 0.07 g/1 1,4-bis-[2-(5 phenyloxazolyl)]-benzene. Quench corrections were made from a quench curve prepared utilizing standard ^{35}S-sodium sulfate (New England Nuclear). The recovery of added ^{35}S-sulfate was 75.0 ± 1.2%.

DNA Determinations: Total DNA in the lung fragments and that released into the incubation medium was determined by the method of Seibert (68). Lung fragments prepared as above were suspended in 3.0 ml Tyrode's medium with and without 100 mM ammonium sulfate and incubated for 30 minutes at 37°C. Aliquots of the supernatant and the whole aqueous homogenates of the fragments were taken for DNA analysis. The difference between the absorbance at 595 and 650 nm of the reaction mixture was determined with a Varian Techtron Model 635 Spectrophotometer (Varian Instrument Division, Palo Alto, Cal.) and compared to a standard curve prepared with calf thymus DNA to determine the amount of DNA present in the aliquot. A typical standard curve is shown in Fig. 2.2.

Lactic Dehydrogenase (LDH) Determinations: The LDH determination was based on the spectrophotometric method of Wroblewski and LaDue (85). Determinations were made with a LDH Diagnostic kit obtained from Sigma Chemical Company (St. Louis, Missouri). ULF were incubated in the presence of 100 mM sodium sulfate and ammonium sulfate for 30 minutes at 37°C. A 0.05 ml aliquot from each flask was pipetted directly into different vials containing 0.2 mg NADH and 2.85 ml 0.1 M potassium phosphate buffer, pH 7.5, mixed and incubated at 25°C. After 20 minutes 0.1 ml 0.02 M sodium pyruvate solution was added to each vial, mixed thoroughly, and the absorbance measured at 340 nm at 30 second intervals for 3 minutes. Measurements were made with a Varian Model 635 spectrophotometer. Total tissue LDH activity was determined by homogenizing the tissue in 5 ml phosphate buffer

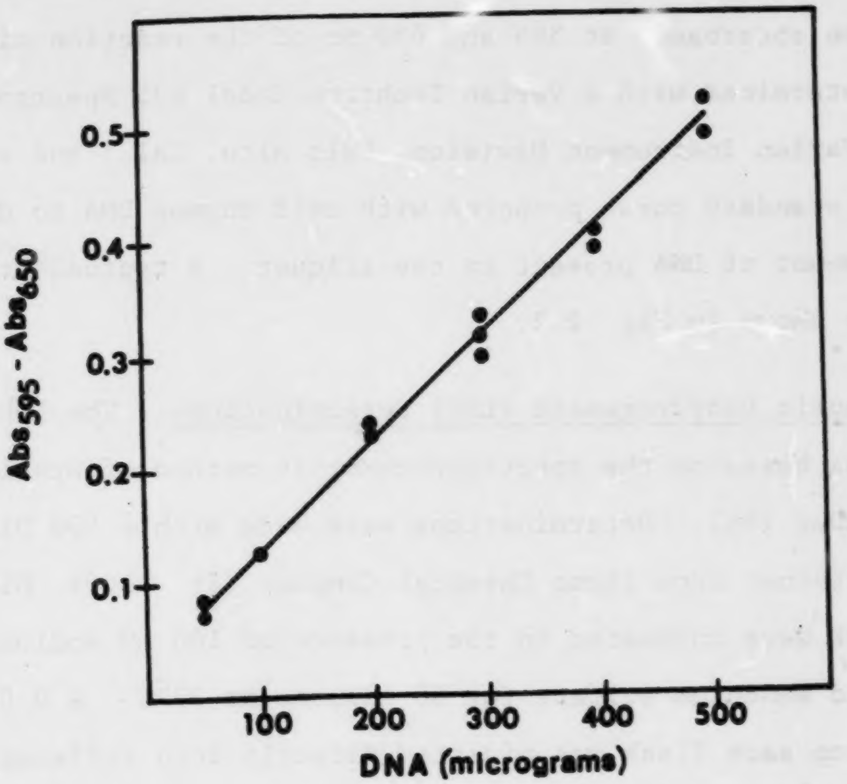


Fig. 2.2. Standard DNA curve determined by the method of Seibert (68)using a stock solution of calf thymus DNA (500 μg/ml).

and essaying as before. LDH activity was calculated from the following formula:

$$\text{LDH activity (units/ml)} = \Delta A/\text{min} \times 20{,}000$$

Metachromatic Titrations of Acridine Orange by Heparin: The absorption spectrum of a 18 μM solution of Acridine Orange dissolved in 1 mM sodium phosphate buffer (pH 6.70, ionic strength = 0.003 μ), was recorded with a Varian Techtron Model 635 spectrophotometer between 560 and 360 nm. Repeated 10 μl additions of a 50 μg/ml solution of sodium heparinate were added and the absorption spectrum recorded. The absorbance at 492 nm of Acridine Orange-heparin solutions was measured in the presence of added concentrations (20-100 mM) NaCl, Na_2SO_4, and $(NH_4)_2SO_4$. Dye concentrations were calculated using the value of $E_{492} = 5.6 \times 10^4$. The number of hypochromatic binding sites per disaccharide repeating unit in each case was calculated from a plot of the molar absorptivity versus volume of heparin solution added (46,72).

Materials: Acetylcholine HCl, Acridine Orange, deoxyribonucleic acid (Calf Thymus, Type V), N^6, O^2-dibutyryl adenosine 3',5'-cyclic monophosphoric acid (monosodium salt), l-epinephrine, sodium heparinate (Grade I), histamine diphosphate, d,l-isoproterenol HCL, l-phenylephrine HCl, and o-phthaldialdehyde were purchased from Sigma Chemical Company (St. Louis, Mo.).

Phloretin was obtained from ICN Pharmaceuticals, Inc. (Cleveland, Ohio). Ovalbumin (2x crystallized) was purchased from Worthington Biochemical Corporation (Freehold, N.J.). Freund's Complete Adjuvant was obtained from Calbiochem (La Jolla, Cal.). ^{35}S-sodium sulfate (947 mCi/mmole) was purchased from New England Nuclear (Boston, Mass.).

RESULTS

Release of Histamine from Unsensitized Lung Fragments by Ammonium Ions and Sulfate Ions: ULF incubated with ammonium sulfate, ammonium nitrate, ammonium acetate, and ammonium chloride, in concentrations of 10-200 mM, released histamine in proportion to the concentration of the salts present (Fig. 2.3). Sodium sulfate and sodium chloride, however, did not release any detectable histamine. Those salts that did release histamine, had varying efficacies. The most efficacious, ammonium sulfate, showed maximal histamine release at concentrations of 100 mM after 30 minutes. The ammonium sulfate mediated release was equal to 97% of the histamine content of unsensitized guinea pig lung. The total histamine present in the unsensitized guinea pig lung was found to be 27.0 ± 3.0 μg per gram of tissue. This value for the histamine content compares favorably with that reported by Shore et.al. (66). The relative potencies of the salts tested are listed in Table 2.1, with ammonium sulfate arbitrarily assigned a value of 100%.

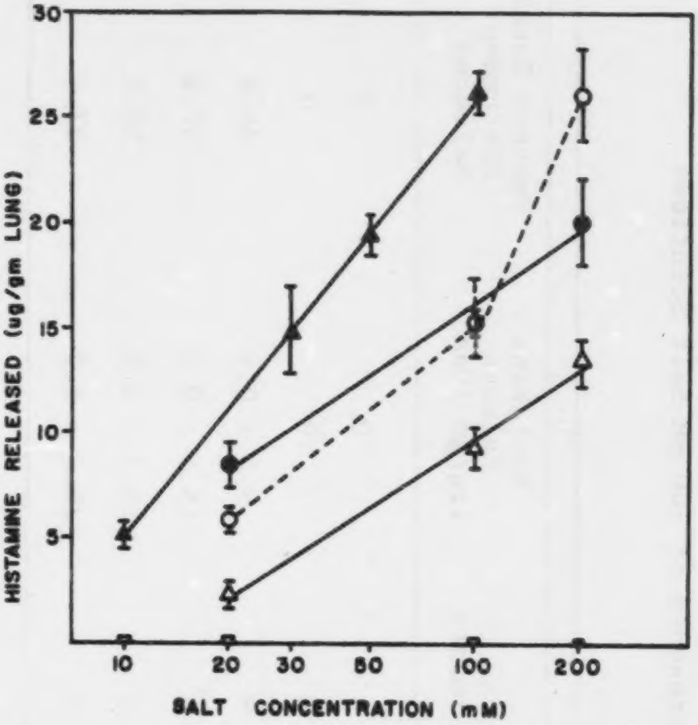


Fig. 2.3. Release of histamine from lung fragments by salts. Each point represents the mean ± s.e. Solid triangles, ammonium sulfate; open circles, ammonium nitrate; solid circles, ammonium acetate, open triangles, ammonium chloride; and open boxes, sodium chloride and sodium sulfate.

TABLE 2.1

Release of Histamine from Lung Fragments in the Presence of 100 mM Salt Solutions

Compound	No. of Experiments	Histamine Release (μg/gm lung)	Percent Total Histamine Released	Relative Potency[a] (%)
Sodium Chloride	6	0	0	0
Sodium Sulfate	6	0	0	0
Ammonium Chloride	9	9.4 ± 0.8	34.8	35.7
Ammonium Acetate	4	15.5 ± 0.3	57.4	58.9
Ammonium Nitrate	5	15.7 ± 1.3	58.1	59.7
Ammonium Sulfate	6	26.3 ± 1.2	97.4	100.0

[a] Ammonium sulfate was arbitrarily assigned a value of 100% since it released approximately 100% of the histamine stores.

Failure of cAMP or cGMP Mediated Systems to Modify Salt Mediated Release of Histamine from Unsensitized Lung Fragments: The possible modulation of salt mediated histamine release in the presence of 10^{-3} isoproterenol is shown in Table 2.2. Isoproterenol had no effect on histamine release.

Similarly, the presence of 10^{-4} M acetylcholine did not significantly change the histamine release by sulfate salts (Table 2.3). Dibutyryl cAMP was preincubated with ULF at a concentration of 10^{-6} M for 5 minutes before the addition of the salts and again no significant change in total histamine release was noted (Table 2.4).

Sensitized guinea pig lung was found to contain 15.8 ± 1.3 μg per gram of tissue. This value is lower than the range of 19-35 μg/g tissue reported by Shore et.al. (66). SLF released histamine in the presence of the antigen, ovalbumin, in a dose response fashion (Fig. 2.4).

Sulfate Ion Uptake by Unsensitized Lung Fragments: The uptake of sulfate ions by ULF in the presence of 10, 50 and 100 mM Na_2SO_4 and $(NH_4)_2SO_4$ is shown in Fig. 2.5. A maximum uptake of 14.5 nanomoles of sulfate ions per mg tissue was observed. The time course for the uptake was similar with both salts, reaching maximal uptake in 30 minutes. A smaller sulfate ion uptake occurred with $(NH_4)_2SO_4$ at 50 and 100 mM concentrations having decreased by 8% and 25% respectively compared to the equivalent concentrations of Na_2SO_4. Sulfate ion uptake was

TABLE 2.2

The Absence of Isoproterenol Mediation on Histamine Release by Sulfate Salts

Compound	No. of Experiments	Concentrations (mM)	Histamine Release in absence of Isoproterenol (10^{-3} M) (μg/gm lung)	Histamine Release in presence of Isoproterenol (10^{-3} M) (μg/gm lung)
Ammonium Chloride	6	100	11.6 ± 2.5	11.1 ± 1.2
Ammonium Sulfate	6	50	20.0 ± 3.8	20.7 ± 4.9
Sodium Sulfate	6	100	0	0

TABLE 2.3

The Absence of Acetylcholine Mediation on Histamine Release by Sulfate Salts

Compound	No. of Experiments	Concentrations (mM)	Histamine Release in absence of Acetylcholine (10^{-4} M) (μg/gm lung)	Histamine Release in presence of Acetylcholine (10^{-4} M) (μg/gm lung)
Ammonium Chloride	6	100	10.2 ± 1.6	8.9 ± 1.3
Ammonium Sulfate	6	50	20.2 ± 4.0	17.9 ± 4.4
Sodium Sulfate	6	100	0	0

TABLE 2.4

The Absence of Dibutyryl cAMP Mediation on Histamine Release by Sulfate Salts

Compound	No. of Experiments	Concentrations (mM)	Histamine Release in absence of Dibutyryl cAMP (10^{-6} M) (μg/gm lung)	Histamine Release in presence of Dibutyryl cAMP (10^{-6} M) (μg/gm lung)
Ammonium Chloride	6	100	8.89 ± 0.96	8.87 ± 0.43
Ammonium Sulfate	6	50	21.31 ± 0.54	18.46 ± 4.60
Sodium Sulfate	6	100	0	0

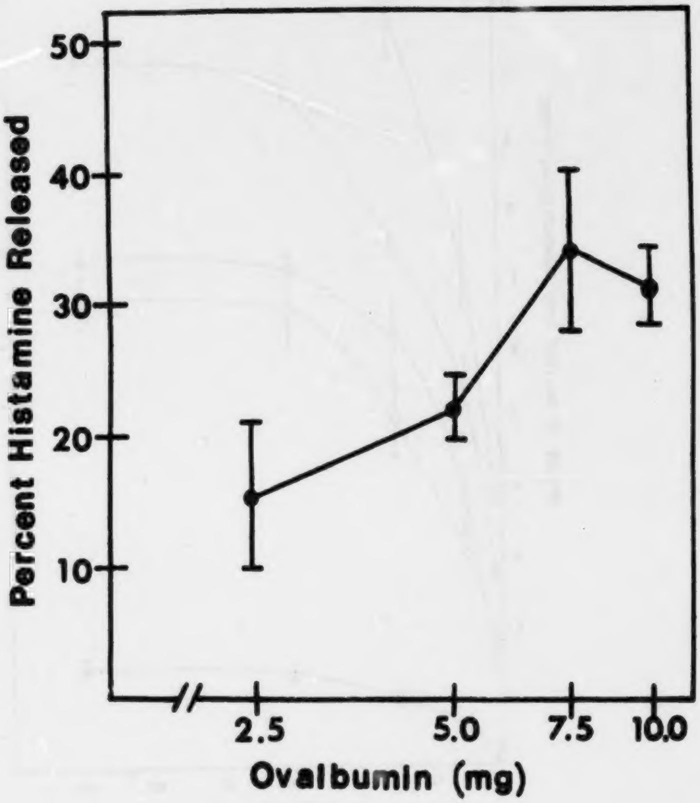


Fig. 2.4 Dose Response release of histamine by antigen (ovalbumin) in sensitized lung fragments. Incubations in the presence of antigen were for 30 minutes in Tyrode's solution at 37°C.

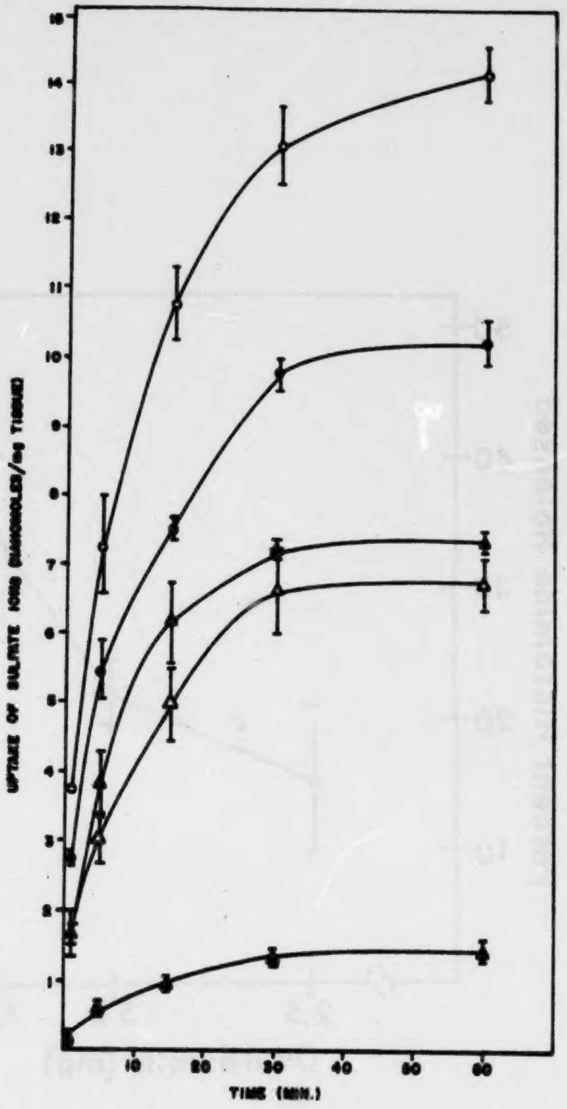


Fig. 2.5 Time course of ^{35}S-sulfate ion uptake by guinea pig lung fragments in the presence of various concentrations of ammonium sulfate and sodium sulfate. Solid triangles, 10 mM Na_2SO_4 and $(NH_4)_2SO_4$. Open triangles, 50 mM $(NH_2)SO_4$. Half solid triangles, 50 mM Na_2SO_4, closed circles, 100 mM $(NH_4)_2SO_4$ and open circles, 100 mM Na_2SO_4.

dependent on the temperature (Table 2.5). Two processes appear to account for the uptake as shown by a plot of the natural logarithm of the uptake versus time. An Arrhenius plot of these data indicated an apparent energy of activation of 3.1 Kcal for the initial kinetic process and 6.1 Kcal for the second.

Sulfate ion uptake was uneffected by NaCN but inhibited by NaF and 2-deoxyglucose (Table 2.6). Even though high concentrations of NaF and 2-deoxyglucose in a glucose-free medium were used complete inhibition could not be achieved.

Isoproterenol, epinephrine, and phenylephrine failed to alter the sulfate ion uptake significantly (Table 2.7). Addition of dibutyryl cAMP also had no effect. Phloretin did not alter sulfate ion uptake.

Sulfate Ion Uptake by Sensitized Lung Fragments: The uptake of sulfate ions by SLF in the presence of antigen, and 50 and 100 mM concentrations of either Na_2SO_4 or $(NH_4)_2SO_4$ is shown in Fig. 2.6. A maximum uptake of 14.6 ± 2.1 nanomoles/mg tissue was observed. Differing from ULF, there was no significant decrease in sulfate ion uptake with 50 mM $(NH_4)_2SO_4$ versus Na_2SO_4 but there was a 15.1% decrease in the presence of 100 mM $(NH_4)_2SO_4$. The uptake was again dependent on temperature (Table 2.8) either in presence or absence of antigen. There was a tendency for less uptake to occur in the presence of the antigen. An Arrhenius plot of the data indicated an apparent energy of activation of 3.3 Kcal/mole for the initial kinetic process and 3.2 Kcal/mole for the second.

TABLE 2.5

Sulfate Ion Uptake in Unsensitized Guinea Pig Lung Fragments

Temperature (^{o}C)	Time (Min)	Sulfate Ion Uptake (picomoles)[a]
0^{o}C	1	6.6 ± 1.1 (6)
	5	16.2 ± 1.7 (6)
	15	45.1 ± 3.3 (7)
	30	57.2 ± 6.6 (5)
	60	67.6 ± 5.0 (4)
22^{o}C	1	11.6 ± 1.1 (7)
	5	28.0 ± 3.3 (4)
	15	51.7 ± 1.6 (6)
	30	67.1 ± 6.0 (5)
	60	86.9 ± 4.4 (8)
37^{o}C	1	13.2 ± 1.7 (5)
	5	30.8 ± 3.3 (4)
	15	67.1 ± 1.0 (7)
	30	84.2 ± 8.2 (6)
	60	122.1 ± 3.3 (5)

[a] The values are expressed as the mean ± s.e.; the number in parentheses is the number of determinations.

TABLE 2.6

Effect of Metabolic Inhibitors on the Uptake of Sulfate Ion by Guinea Pig Lung Slices[a]

Additions	Per Cent Uptake of ^{35}-S-Sulfate
None	100
Sodium Fluoride (10^{-3} M)	85 ± 4
Sodium Cyanide (10^{-3} M)	111 ± 3
2-Deoxyglucose (10^{-2} M)	85 ± 4

[a] Lung slices were preincubated for 10 min with the inhibitors prior to the addition of 1 μCi ^{35}S-Na_2SO_4. Incubations were carried out in Tyrode's solution for 30 min at 37°C. Each value is the mean ± s.e. of 8 experiments.

TABLE 2.7

Failure of Pharmacological Agents to Modulate the Uptake of Sulfate Ion by Guinea Pig Lung Slices[a]

Additions	Per Cent Uptake of ^{35}S-Sulfate
Phenylephrine	101 ± 6
Epinephrine	95 ± 16
Isoproterenol	104 ± 10
Dibutyryl Cyclic AMP	96 ± 9
Phloretin	102 ± 17

[a] Lung slices were preincubated for 10 min with the pharmacological agents (10^{-5} M) prior to the addition of 1 μCi $^{35}S\text{-}Na_2SO_4$. Incubations were carried out in Tyrode's solution for 30 min at 37°C. Each value is the mean ± s.e. of 8 experiments.

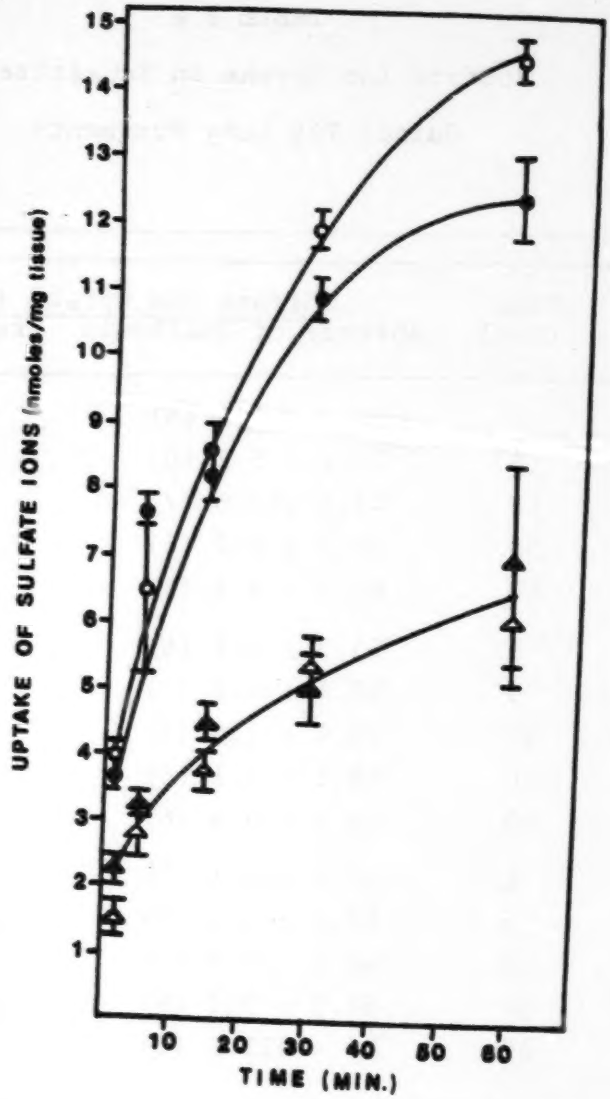


Fig. 2.6. Time Course of ^{35}S-Sulfate ion uptake by sensitized guinea pig lung fragments in the presence of various concentrations of sodium sulfate and ammonium sulfate. Open triangles, 50 mM sodium sulfate; solid triangles, 50 mM ammonium sulfate; open circles 100 mM sodium sulfate; and solid circles, 100 mM ammonium sulfate.

TABLE 2.8

Sulfate Ion Uptake in Sensitized Guinea Pig Lung Fragments

Temperature (°C)	Time (min)	Sulfate Ion Uptake (Picomoles)[a]	
		Absence of Ovalbumin	Presence of Ovalbumin
0°	1	15.7 ± 1.5 (6)	12.4 ± 1.1 (7)
	5	23.2 ± 5.2 (6)	20.5 ± 5.9 (7)
	15	42.2 ±10.8 (7)	35.1 ± 4.3 (7)
	30	53.1 ± 8.2 (6)	45.5 ± 2.6 (8)
	60	60.7 ± 8.5 (5)	55.6 ± 3.1 (6)
22°	1	14.5 ± 0.9 (6)	13.7 ± 0.5 (7)
	5	39.9 ± 1.2 (7)	27.0 ± 1.9 (6)
	15	48.4 ± 1.6 (8)	48.2 ± 2.2 (7)
	30	60.1 ± 5.1 (6)	57.5 ± 4.5 (7)
	60	99.7 ± 6.4 (6)	78.0 ± 4.6 (7)
37°	1	17.4 ± 2.1 (5)	8.6 ± 1.1 (7)
	5	20.6 ± 1.2 (7)	39.3 ± 1.9 (6)
	15	46.8 ± 4.4 (5)	55.8 ± 1.7 (7)
	30	64.0 ± 5.2 (6)	71.5 ± 4.8 (7)
	60	96.4 ±11.2 (6)	89.0 ±11.0 (9)

[a] The values are expressed as the mean ± s.e.; the number in parentheses is the number of determinations.

Sulfate ion uptake was uneffected by 2-deoxyglucose in a glucose-free medium, but inhibited by NaF and NaCN. Even though high concentrations were used, complete inhibition could not be achieved (Table 2.9).

Isoproterenol, epinephrine, phenylephrine, acetylcholine and dibutyryl cAMP modulated the anaphylactic release of histamine as reported previously (44,50,57), but failed to alter sulfate ion uptake significantly. Phloretin also failed to alter the sulfate uptake but decreased histamine release. The results are shown in Table 2.9.

Dependence of Metachromasia of Acridine Orange on Ionic Strength: Acridine Orange solutions exhibited metachromasia in the presence of heparin (Fig. 2.7), which was dependent on the ionic strength of the solution. Addition of $(NH_4)_2SO_4$, NH_4Cl, Na_2SO_4, and NaCl decreased the metachromasia of the Acridine Orange-heparin solutions an equivalent amount at the same ionic strength (Fig. 2.8). The metachromatic titration of Acridine Orange with heparin in the presence of these salts is shown in Fig. 2.9. Equivalent end points were found under all conditions. While the affinity of heparin for Acridine Orange decreases with increasing ionic strength, as shown by the decrease in metachromasia with increasing ionic strength, the number of binding sites per disaccharide unit remains constant (Table 2.10).

TABLE 2.9

Effects of Pharmacological Agents on the Anaphylactic Release of Histamine and Sulfate Ion Uptake in Sensitized Guinea Pig Lung Fragments[a]

Additions	Concentration	No. of Determinations	Percent Release of Histamine[b]	Percent Uptake of ^{35}S-Sulfate Ions[b]
Control	------	20	100	100
2-Deoxyglucose	10^{-3} M	17	100.0 ± 5.1	101.8 ± 4.5
Sodium Cyanide	10^{-3} M	17	60.1 ± 8.1**	89.6 ± 5.7**
Sodium Fluoride	10^{-3} M	16	97.2 ± 7.8	88.5 ± 4.0**
Acetylcholine	10^{-5} M	17	110.2 ± 6.4**	93.6 ± 5.1
Dibutyryl cAMP	10^{-5} M	18	95.3 ± 3.1	96.6 ± 2.5
Epinephrine	10^{-5} M	19	79.0 ± 5.5**	97.4 ± 4.6
Isoproterenol	10^{-5} M	18	64.5 ± 4.8**	99.7 ± 5.6
Phenylephrine	10^{-5} M	16	92.4 ± 5.1	97.6 ± 3.2
Phloretin	10^{-5} M	15	84.6 ± 4.3**	98.8 ± 5.2

[a] All preincubations with the agents were for 10 minutes followed by the addition of 5 mg of Ovalbumin. Ten minutes later radioactivity was added and 30 minutes later the supernatant was assayed for histamine and the fragments oxygen combusted to determine ^{35}S-radioactivity.

[b] The values are expressed as the mean ± s.e.

** Values differ significantly from control at $p < 0.05$

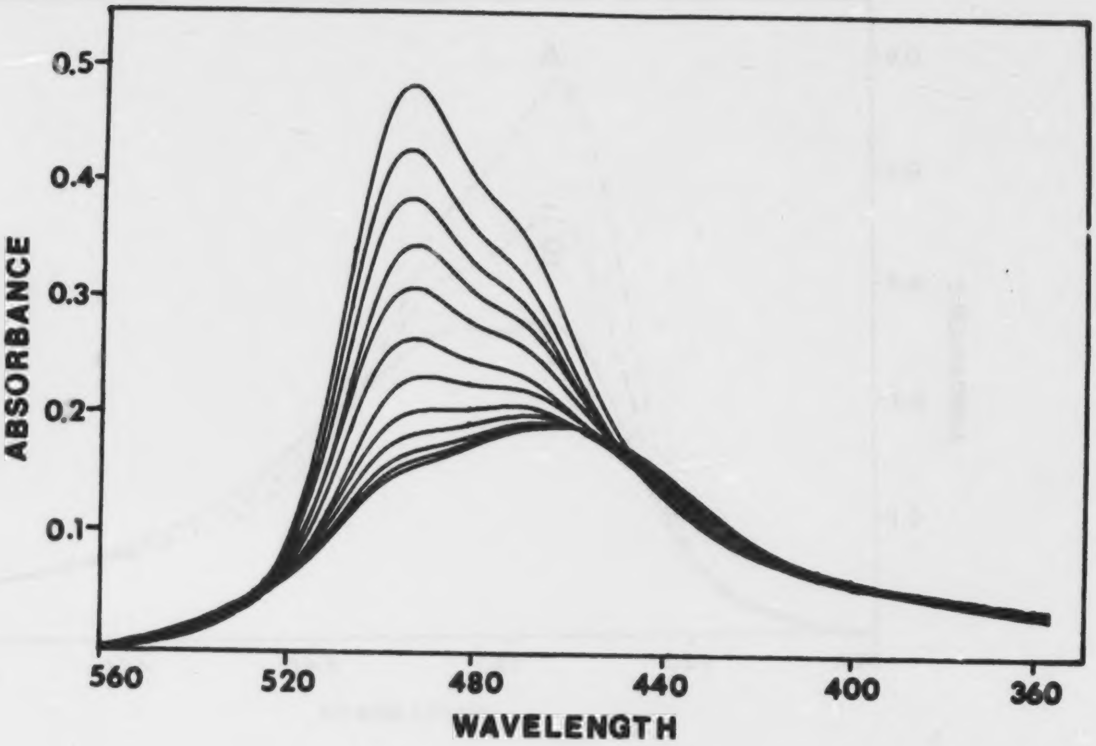


Fig. 2.7. Metachromatic titration of a solution of Acridine Orange (0.003μ) with 10 microliter additions of heparin solution (50 ng/microliter). Top curve is absorption spectrum of Acridine Orange alone. Each subsequent curve represents the metachromatic shift of the spectrum upon the addition of 10 microliters of the heparin solution.

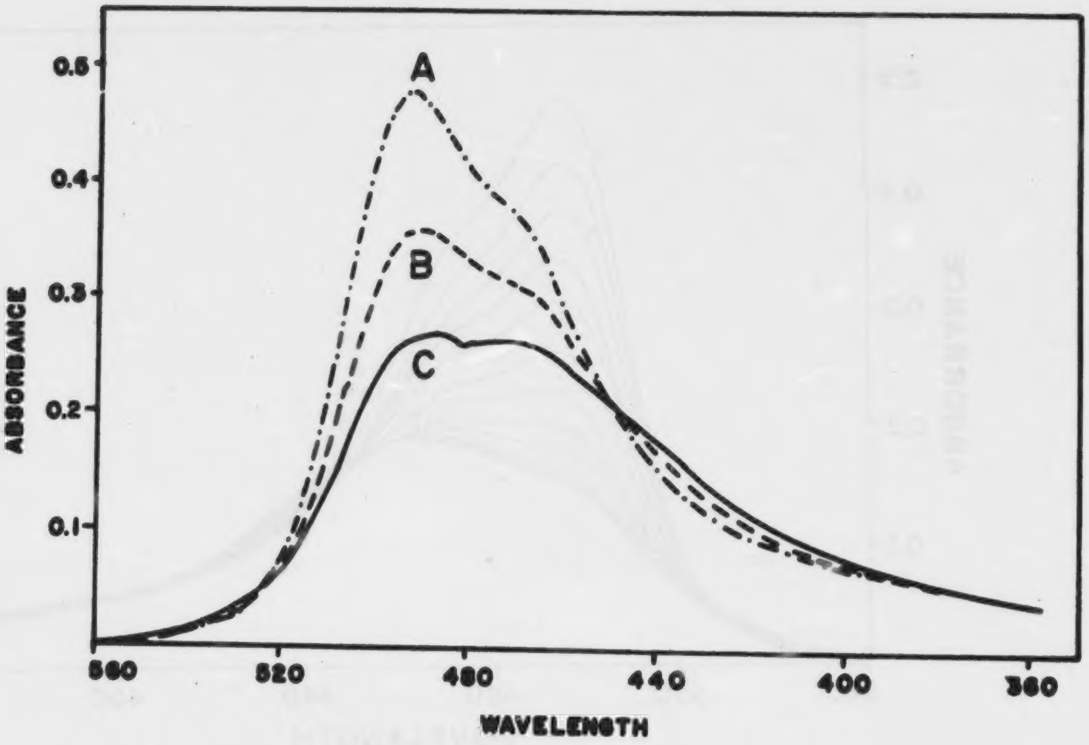


Fig. 2.8. Absorption spectra of (A) Acridine Orange (0.003μ), (B) 3.0 μg sodium heparinate added to Acridine Orange (0.003μ) in presence of 0.063μ of the various salts tested, (C) 3.0 μg sodium heparinate added to Acridine Orange at 0.003μ.

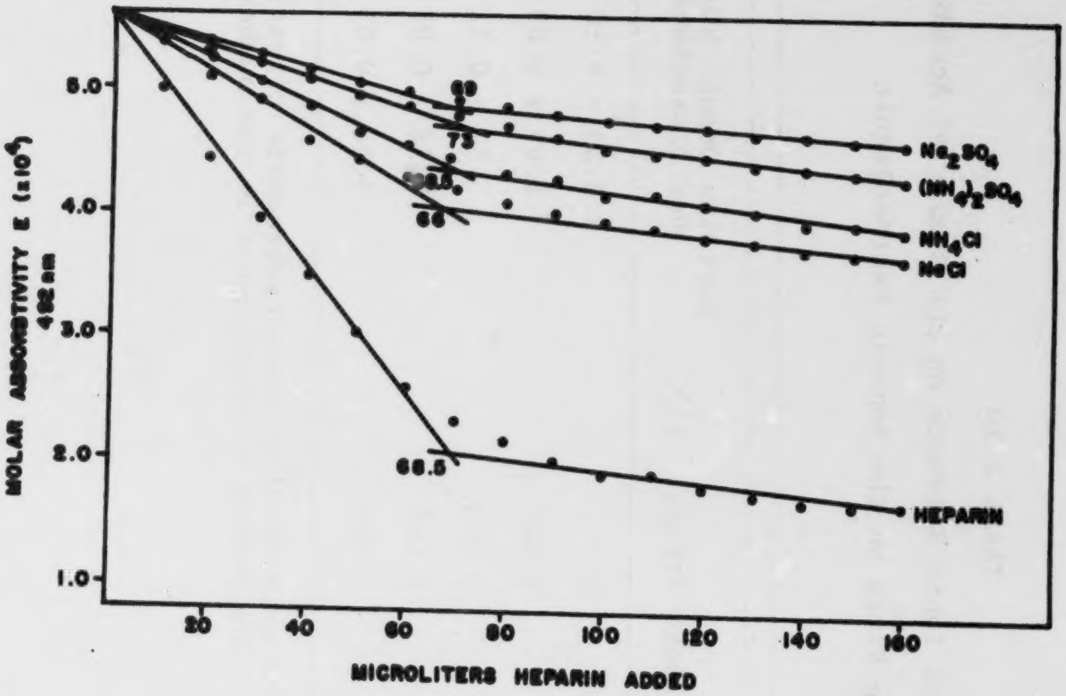


Fig. 2.9. Metachromatic Titration of Acridine Orange by Heparin Varying Ionic Strengths and Salt Compositions. Heparin denotes the titration at 0.003 μ; Na_2SO_4 0.060 μ; $(NH_4)_2SO_4$ 0.060 μ; NH_4Cl 0.020 μ; NaCl 0.020 μ. The point of inflection is noted in μl of added heparin solution (50 ng/μl). See text for experimental details.

TABLE 2.10

Absence of an Effect of Ionic Strength on the Number of Acridine Orange Binding Sites on the Heparin Macromolecule

Additions to Acridine Orange Solution	Ionic Strength (μ)	Acridine Orange Binding Sites[a] per Dissacharide Unit
Heparin	0	3.53 ± 0.10 (4)
Heparin + NaCl	0.020	3.69 ± 0.03 (3)
Heparin + NH_4Cl	0.020	3.37 ± 0.11 (4)
Heparin + $(NH_4)_2SO_4$	0.060	3.58 ± 0.05 (4)
Heparin + Na_2SO_4	0.060	3.58 ± 0.05 (4)

[a] Acridine Orange binding sites were obtained by metachromatic titration and are recorded as the mean ± s.e.; the number in parentheses is the number of determinations.

Absence of Cell Lysis in the Presence of Salts: Cell lysis does not appear to contribute significantly to either salt mediated histamine release or the decrease in sulfate ion uptake at high concentrations of $(NH_4)_2SO_4$. All of the DNA originally present in the tissue slice could be found in the fragments (4.7 ± 0.2 mg DNA/gm vs. 4.8 ± 0.2 mg/gm). LDH release into the incubation medium in the presence of 100 mM Na_2SO_4 or $(NH_4)_2SO_4$ did not significantly differ from the control incubated in Tyrode's alone (Table 2.11).

SUMMARY AND CONCLUDING REMARKS

Because sulfate residues exist mainly as particles in the atmosphere, the local alveolar concentration resulting from the dissolution of a respirable particulate could be high. It appears that ammonium sulfate, in the concentration of 100 mM, releases approximately 100% of the stored histamine in the guinea pig lung. An equal ammonium ion concentration (200 mM ammonium chloride) released only half of the histamine stores. Since lung fragments in the presence of 200 mM sodium chloride exhibited no detectable release of histamine, the histamine released by ammonium chloride is ascribed to the ammonium ion. Similarly, the difference observed in histamine released between 100 mM ammonium sulfate and 200 mM ammonium chloride must be some function of the sulfate anion. The sulfate ion, *per se*, has no inherent effect alone, since sodium sulfate

TABLE 2.11

Release of LDH from Unsensitized Lung Fragments

Treatment	No. of Determinations	LDH Activity Released[a] (units/mg tissue)
Homogenate	5	98.9 ± 9.40
Tyrode's solution alone	4	3.02 ± 0.36
Tyrode's solution containing 100 mM Na_2SO_4	8	3.23 ± 0.17
Tyrode's solution containing 100 mM $(NH_4)_2SO_4$	8	3.28 ± 0.20

[a] The values are expressed as the mean ± s.e.

at concentrations ranging from 10 to 200 mM caused no histamine release. The presence of ammonium ion seemed to be a necessary factor for histamine release. Ammonium nitrate and acetate were of intermediary potency in histamine release.

These experiments demonstrate that the histamine released by ammonium and sulfate ions in ULF cannot be modulated by either cAMP or cGMP, since isoproterenol, acetylcholine and dibutyryl cAMP failed to influence histamine release.

Studies in SLF demonstrated the known phenomenon of the ability of the cAMP and cGMP systems to modulate antigen-antibody release of histamine. Acetylcholine, epinephrine and isoproterenol all had significant effects ($p < .05$) on histamine release in accordance with previous reports (44,57,58). Dibutyryl cAMP and phenylephrine failed to have significant effects, although reported to be capable of modulation (57,58). An unexpected observation was the ability of phloretin to decrease the release of histamine.

Release of histamine through lysis of the mast cells is not likely since neither DNA nor LDH, a cytoplasmic enzyme, were released into the supernatant in the presence of ammonium sulfate as compared to control. Total DNA in the fragments also remained constant in the presence of concentrations of ammonium sulfate known to release histamine. Thus, the observed histamine release is likely to be a degranulation phenomenon.

The intracellular sulfate ion space in ULF and SLF decreased in the presence of $(NH_4)_2SO_4$, the decrease in the intracellular sulfate ion space is probably associated with the degranulation process. The intracellular sulfate space in SLF was significantly altered in the presence of 100 mM $(NH_4)_2SO_4$.

In both experimental systems, drugs capable of modulating the cAMP and cGMP systems failed to alter the sulfate ion uptake. The sulfate ion transport system does not appear to be highly dependent on the availability of metabolic sources of energy. At high concentrations of potent metabolic inhibitors only partial inhibition of sulfate ion uptake was observed. In the case of ULF, both sodium fluoride and 2-deoxyglucose showed inhibition and in SLF sodium fluoride and sodium cyanide had effects. Phloretin has been reported to inhibit chloride and sulfate uptake by human red blood cells (83), however, phloretin had no effect on the sulfate ion uptake by the lung fragments.

Data presented here, concurs with the observations of others (72), that the metachromasia associated with Acridine Orange binding to heparin is a function of ionic strength. The number of Acridine Orange binding sites found in our experiments (3.53 ± 0.10 binding sites per disaccharide unit) corresponds favorably with that reported by Lagunoff (46) of 3.31 ± 0.09 binding sites per disaccharide unit. The number of binding sites remained constant with increasing ionic strength. Stone and Bradley (72) and Lagunoff (46) suggest that the binding sites for Acridine Orange and histamine on the heparin macromolecule are identical. Since we observed a decrease in the extent of Acridine Orange binding to the heparin macromolecule with increasing ionic strength, a local increase in the ionic strength within the granule is likely to cause displacement of histamine bound to heparin. Since the mast cell granule is freely permeable to the external ionic environment, intracellular uptake of ammonium or sulfate ions could result in the displacement of bound histamine.

SECTION 5

ABSORPTION OF SULFATE ION IN THE ISOLATED, VENTILATED, AND PERFUSED RAT LUNG

INTRODUCTION

The isolated, ventilated and perfused lung (IVPL) would seem to be most similar to the lung in vivo but it is technically difficult to maintain free of edema with normal perfusion rates. However, when compared with other in vitro preparations it has several merits.

The tissue slice method utilized in the previous chapter has certain intrinsic shortcomings. The lung is damaged during the slicing procedure, substrates are delivered to the cut edge of the tissue or alveolar epithelium rather than through the capillaries, and oxygen must be supplied through a liquid medium to the edge of the slice instead of through gas in the air spaces and physiological medium in the capillaries.

The IVPL has been used extensively to study metabolism and uptake by the lung vasculature of numerous compounds. The techniques employed for the IVPL are modifications of those developed by Niemeier and Bingham (55) and Rosenbloom and Bass (62).

Gassenheimer and Rhodes (31) studying the influence of ventilation frequency on glucose and palmitate uptake and metabolism in the rat IVPL, found that oxidation of glucose reached a maximum at 70 cycles per minute and decreased thereafter to 215 cycles per minute. Palmitate incorporation into the lung lipids increased non-linearly with frequency. Angiotensin I in the perfusate is converted to Angiotensin II in the dog and rat IVPL (25,64). Radioactivity is not retained by the lungs and has the same volume distribution and mean transit time as blue dextran, a compound unlikely to leave the intravascular space (64), suggesting that angiotensin is hydrolyzed by enzymes located on the luminal surface of pulmonary endothelial cells. Prostaglandin $F_{1\alpha}$ is largely eliminated from the circulation during a single passage through the pulmonary vascular bed and the volume of distribution and the mean transit time are greater than blue dextran (63). Metabolism therefore requires cellular uptake.

The effect of CO_2 concentration on phospholipid metabolism has been investigated by Longmore et.al.(51). Glucose was found to be a precursor of the palmitate of phosphatidylcholine. The CO_2 concentration of the perfusion was found to effect the incorporation of glucose in palmitate.

Both serotonin and norepinephrine are taken up by the lung vasculature in the rabbit (10,40,41) and rat (43,54) IVPL. Willis and Kratzing (84) utilized the rat IVPL to determine the location of ascorbic acid in the lung. Ascorbic acid was not present in the effluent after perfusion of the pulmonary circulation, but 30% of the total present in the lung was recovered by washing out the air spaces. They concluded that up to 50% of the ascorbic acid is present in the fluid lining of the air spaces.

The IVPL has never been employed to study the absorption of compounds in the airways by the lung vasculature. The removal of organic compounds from the airway in vivo has been studied by Schanker and his colleagues. Corticosteroids (14) and antibiotics (13) are removed by simple diffusion with half lives from 1.9 to 33.0 minutes. The removal of charged organic molecules also occurs by simple diffusion with half lives of 1 to 70 minutes (23). An exception is phenol red which is removed by facilitated transport with a half life of 20 minutes (24).

Only a few studies of the movement of simple anions across the lung have appeared. Gatzy (32) proposed that chloride ion transport accounts for the potential difference found across the alveolar membranes of the amphibian lung. Chloride ion transport also is found in other epithelia such as the toad bladder (27) and frog epithelium (39). Using the permeability coefficient of sodium ion, the $t_{\frac{1}{2}}$ for sulfate ion transport in the turtle lung can be calculated as 27.8 minutes (19). Sulfate ion transport in mammalian lungs has not been reported previously. In this chapter the uptake of sulfate ions from the airways of the isolated, ventilated, and perfused rat lung, in the absence and presence of various ions is described. The release of histamine and lactic dehydrogenase (LDH), a cytoplasmic enzyme, by the ions is also presented.

METHODS

Preparation of the Isolated, Ventilated, and Perfused Lung: Female Sprague Dawley rats, weighing 250-300 g, were anesthetized with pentobarbital (35 mg/kg). The isolated and perfused rat lung was prepared by a modification of the technique of Niemeier and Bingham (55). The trachea was isolated through a midline incision and a cannula (PE 240) was inserted. The aorta and inferior vena cava are severed to allow exsanguination of the animal. The lungs and heart are exposed through a midline sternotomy and the rib cage retracted. The pulmonary artery was cannulated through a small incision made in the right ventricle with a cannula (PE 200) filled with modified Tyrode's solution. The entire right ventricle and right atrium, together with most of the left ventricle were removed. The modified Tyrode's solution (pH 7.35, 37°C) contained 35.0 g/l polyvinylpyrrolidone (average molecular weight 40,000) to maintain a physiological oncotic pressure. Perfusion of the lungs was started immediately to remove any remaining blood in the pulmonary circulation. The perfusion rate was maintained at a constant 2.0 ± 0.1 ml per minute.

The lungs were removed from the animal and suspended by the tracheal cannula in an artificial thorax (25°C) where respiration was maintained mechanically by an alternating negative pressure (-3 to -15 cm of water). Positive pressure ventilation may lead to edema, destruction of alveolar septae by over-inflation, and is reported to contribute to alveolar collapse and progressive atelectasis (21,42,82). The respiratory rate was kept at 90 inspirations per minute. A Harvard small animal respirator (Harvard Apparatus, Millis, Mass.), was used to provide an alternating +6 and -6 cm H_2O pressure, superimposed over a background -9 cm of H_2O within the chamber, generated by a vacuum pump. Pressure was monitored by a Magnahelic gauge. Respiratory volume was monitored by a Grass volumetric pressure transducer connected to the tracheal cannula. The system for ventilating and perfusing the lung is shown in Fig. 3.1.

Determination of the Relative Distribution of an Intratracheal Injection: A study was undertaken to determine the relative distribution within the airways of a single intratracheal injection of 100 μl of isotonic (0.3 M) sucrose. Following injections of 4 μCi of $^{35}S\text{-}Na_2SO_4$, perfusion was continued for 1 minute and the ^{35}S-radioactivity within each lobe was determined by flask oxygen combustion as described previously.

Results are expressed as dpm per mg of wet lung tissue. All subsequent experiments were carried out with a single injection of 100 μl of 0.3 M sucrose.

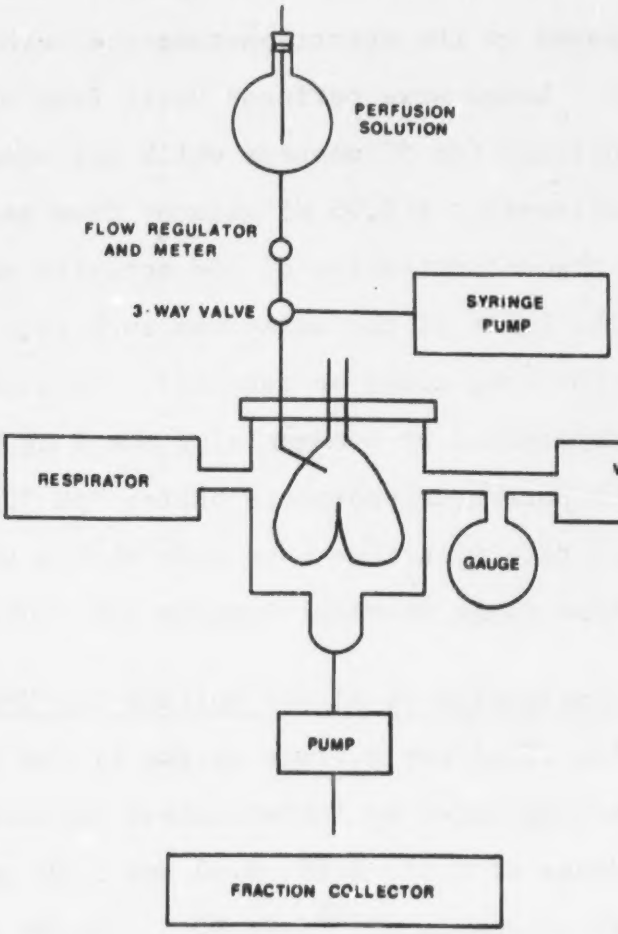

PERFUSION SOLUTION
FLOW REGULATOR AND METER
3-WAY VALVE
SYRINGE PUMP
RESPIRATOR
VACUUM PUMP
GAUGE
PUMP
FRACTION COLLECTOR

Fig. 3.1. Schematic Diagram of the Isolated, Ventilated and Perfused Lung Preparation.

Possible Release of LDH by Lung Perfusion: The possible release of LDH during perfusion of the pulmonary circulation with modified Tyrode's solution was investigated. The LDH determination was based on the spectrophotometric method of Wroblewski and LaDue (85). Lungs were perfused until free of blood. Perfusion was continued for 30 minutes while one minute aliquots (2 ml) were collected. A 0.05 ml aliquot from each minute sample was taken for the determination of LDH activity as described in Chapter II. The limit of the assay was such that 29 IU/min released from the lung could be detected. Total tissue LDH activity was determined by homogenizing the lung tissue in 5 ml ice cold 0.10 M potassium phosphate buffer (pH 7.5) and assaying as before. All determinations were made with a LDH Diagnostic Kit obtained from Sigma Chemical Company (St. Louis, Mo.).

Kinetic Characterization of Airway Sulfate Ion Uptake: Kinetic characterization of airway sulfate uptake by the pulmonary circulation was accomplished by intratracheal injection of total sulfate salt doses of 0.01, 0.05, 0.10 and 1.00 μmole of sulfate containing 0.65 μCi/mmole of $^{35}S\text{-}Na_2SO_4$. The pH of the solutions was adjusted to 7.40. Aliquots were collected from the lung effluent each minute (2 ml) and ^{35}S-radioactivity determined by liquid scintillation counting. Results are expressed as the logarithm of the percent sulfate ions unabsorbed by the lung versus time.

Investigation of Possible Sulfate Ion Uptake from the Vasculature into the Lung: To investigate the possibility of sulfate ion uptake from the vasculature into the lung, ^{35}S-sodium sulfate dissolved in modified Tyrode's solution was perfused into the lung at 1.6 µCi/min (256 ng/min) at a flow rate of 2 ml/min for 10 min using a syringe pump (Fig 3.1). The perfusion solution also contained blue dextran, 5 mg/ml. Blue dextran (ave. mol. wt. 2,000,000) was used as a compound unlikely to leave the vascular space during a single circulation and has been used as a basis for calculating apparent mean transit times and volume of distribution within the lungs (18). The perfusion was continued for another 20 minutes with the modified Tyrode's solution alone. The venous effluent was collected every tenth of a minute and 3 ml distilled H_2O were added to each sample. The blue dextran content was estimated from the absorbance at 600 nm. Aliquots of each sample were taken for the determination of ^{35}S-radioactivity.

Modulation of Sulfate Ion Absorption from the Airways by the Counter Cation: To investigate the absorption of sulfate ions in the presence of other cations, an unlabeled chloride salt of the cation in question was dissolved in the isotonic sucrose solution together with the ^{35}S-sodium sulfate (specific activity - 0.64 µCi/mmole) prior to injection. Injections of 0.1 ml isotonic sucrose solution were used containing 0.1 µmole ^{35}S-sodium sulfate and either 0.1, 1.0 or 10.0 µmole of chloride salt.

Absorption was allowed to proceed for 30 minutes. Aliquots (2 ml) were collected from the lung effluent each minute and the ^{35}S-radioactivity determined. Chloride salts studied in this manner were ammonium, cadmium, cobaltous, ferric, manganous, mercuric, nickelous, and zinc.

Possible Release of LDH, Prostaglandins and Histamine by Sulfate Salts: The possible release of LDH into the lung effluent was determined, after the injection of 0.1 ml isotonic sucrose solution containing 1.0 μmole sodium sulfate alone and in combination with 1.0 μmole of the chloride salts tested separately.

The possibility of release of prostaglandins by the salts was also investigated. The lung effluent was mixed with a stream of modified Tyrode's medium containing mepyramine maleate (4.2×10^{-7} g/ml), atropine sulfate (9.6×10^{-7} g/ml), methysergide (8.3×10^{-7} g/ml), propranolol (1.3×10^{-5} g/ml), and aspirin (6.4×10^{-4} g/ml). This mixture was then superfused onto a rat stomach strip prepared according to the method of Vane (81). With the inhibitor mixture, the rat stomach strip response is specific for prostaglandins. The release of histamine by the salts was also determined spectrophotoflurometrically by the method of Shore (66).

Bronchoconstriction Mediated by Certain Sulfate Salts: Bronchoconstriction by sulfate salts was investigated by monitoring the respiratory volume prior to and after intratracheal administration of 0.1 ml of 1 μmole of various salts in isotonic sucrose solution. The salts tested in this fashion were Na_2SO_4, NH_4Cl, and $(NH_4)_2SO_4$. The effect on intratracheal injection of 14 μg histamine in 0.1 ml isotonic sucrose solution on respiratory volume was examined. With those salts decreasing the respiratory volume, another experiment was performed perfusing the lung with Tyrode's medium containing 1.5×10^{-5} mepyramine maleate (H-1 antihistamine) to determine if the bronchoconstriction could be prevented.

Absorption of ^3H_2O by the Rat IVPL: Removal of water from the airways by the pulmonary circulation was determined by intratracheal injection of 100 μl of ^3H_2O (1 μCi/mmole) containing 0.3 M sucrose. Samples were collected each minute, as before, from the lung effluent and ^3H-radioactivity determined by liquid scintillation counting. The effect of 0.10 μmole Na_2SO_4 and 0.10 μmole $(NH_4)_2SO_4$ on removal of water from the airways was also examined by addition of these salts to the ^3H_2O. Each curve is the mean of three experiments.

Materials: Blue dextran 2000 was obtained from Pharmacia Fine Chemicals. ^3H_2O and ^{35}S-sodium sulfate were purchased from New England Nuclear.

RESULTS

Distribution of an Intratracheal Injection in the Rat IVPL: In order to determine the reproducibility of intratracheal instillation as a route of exposure of the lung to sulfate salts, a single injection of 100 μl of isotonic sucrose containing 4 μCi of $^{35}S\text{-}Na_2SO_4$ was given and the perfusion and ventilation were continued for one minute. The lung was then removed from the apparatus, divided into five portions and the ^{35}S-radioactivity was determined in each lobe (Table 3.1). The right lower and left upper lobes of the lung contained the highest radioactivity while the right middle lobe contained the lowest. While the distribution of radioactivity was not uniform, intratracheal instillation did provide a reproducible method of exposure.

Absence of Release of Significant Quantities of LDH by Perfusion: Figure 3.2 depicts the LDH activity released by perfusion of the lung vasculature with modified Tyrode's solution. The amount released never exceeded 40 IU/min. This release is insignificant when compared to the total LDH activity of 63.8 ± 9.9 IU/mg tissue wet weight. An average rat lung would therefore contain approximately 84,000 IU.

Kinetics of Sulfate Ion Removal from the Airways: Figure 3.3 shows a semilogarithmic plot of the removal of doses of 0.01 and 0.10 μmole ^{35}S-sulfate ions from the rat IVPL following intratracheal administration. From such curves the initial first order rate constants and $t_{\frac{1}{2}}$ were calculated. A summary of these data are presented in Table 3.2. The rate of removal of 0.05 μmole Na_2SO_4 or greater was constant. The average $t_{\frac{1}{2}}$ for sulfate ion

TABLE 3.1

Distribution of Intratracheal Injection of 0.1 ml Isotonic Sucrose Containing 4 μCi of ^{35}S-Sodium Sulfate

Lobe of Lung	Weight (gm)	dpm/mg wet lung tissue*
Right upper	0.111 ± 0.010	2456 ± 758
Right middle	0.151 ± 0.012	1344 ± 303
Right lower	0.368 ± 0.022	5319 ± 1407
Left upper	0.155 ± 0.012	6462 ± 1024
Left lower	0.438 ± 0.025	3601 ± 1029
Total lung wg. -	1.210 ± 0.060	

*Expressed as mean ± s.e. of 10 experiments.

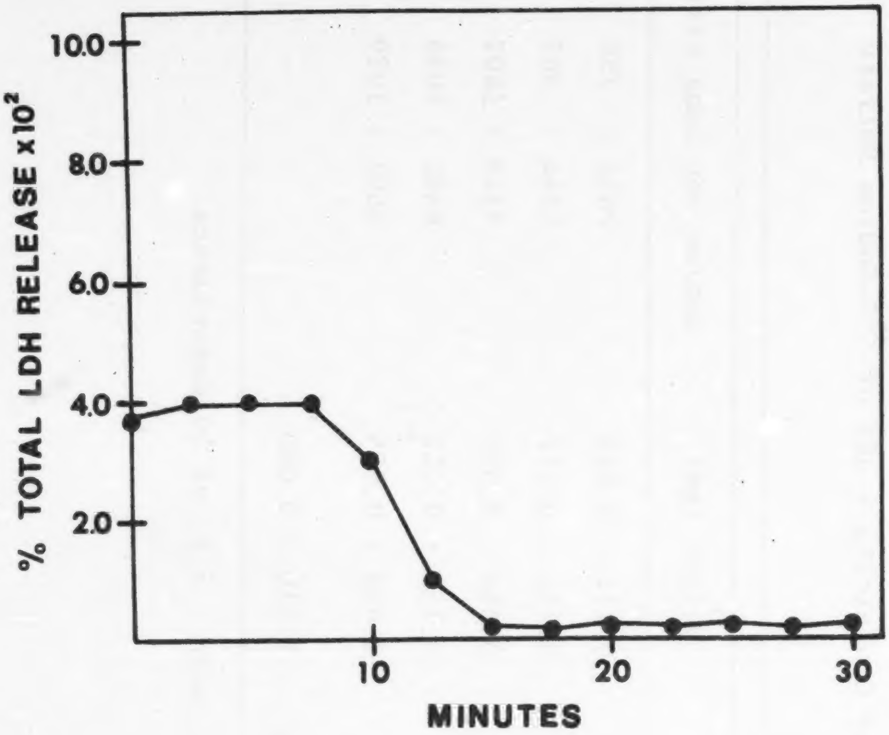


Fig. 3.2. Absence of significant amounts of LDH in effluent of isolated, ventilated and perfused rat lung. LDH was detected by the method of Wroblewski and LaDue (85). Percent total LDH released is based on an average activity per lung of 84,000 IU.

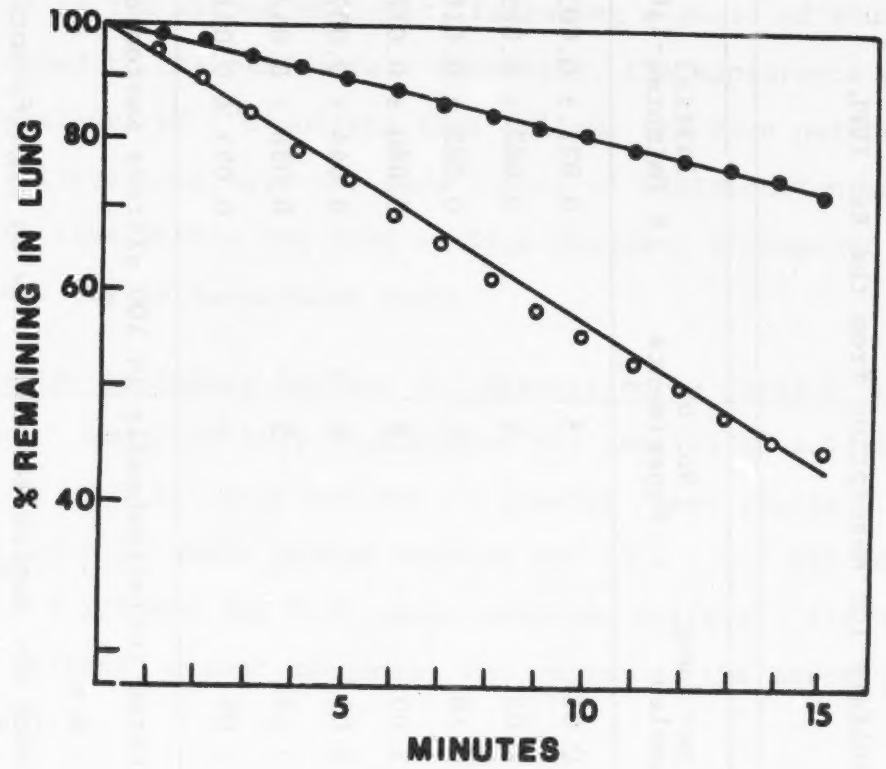


Fig. 3.3. Removal of ^{35}S-sulfate ions from the isolated, ventilated and perfused rat lung. Solid circles represent 0.01 μmole sodium sulfate; open circles 0.1 μmole.

TABLE 3.2

Effect of Ammonium Ion on the Kinetics of Sulfate Ion Absorption from the Rat IVPL

Compound[a]	Dose per Lung (μmole)	No. of Experiments	Initial K (minutes^{-1})[b]	$t_{\frac{1}{2}}$ (minutes)[b]
Sodium Sulfate	0.01	3	0.021 ± 0.003	33.0 ± 4.7**
	0.05	4	0.082 ± 0.025	8.4 ± 2.6
	0.10	11	0.085 ± 0.016	8.1 ± 1.3[c]
	1.00	5	0.080 ± 0.018	8.7 ± 2.0[d]
Ammonium Sulfate	0.01	4	0.044 ± 0.007	15.7 ± 2.5**
	0.10	3	0.086 ± 0.047	8.1 ± 3.5[c]
	1.00	4	0.093 ± 0.031	7.5 ± 2.5[d]

[a] All solutions were administered intratracheally in 100 μliters isotonic sucrose, pH 7.4.

[b] Values are expressed Mean ± s.e.

[c and d] Paired "t" test between the indicated means show no significant difference at 0.05 level of confidence.

** Statistical significant difference between sodium sulfate and ammonium sulfate, $p < 0.01$.

removal by the rat IVPL for doses of 0.05 μmole and larger is 8.4 ± 1.8 minutes (20 determinations).

Absence of Pulmonary Vascular Uptake of ^{35}S-Sulfate Ions: Figure 3.4 shows the appearance and then disappearance of blue dextran in the venous effluent, following a pulse of blue dextran added to the perfusate. Similarly, the appearance and then disappearance of ^{35}S-sulfate ions follows the same pattern. Thus sulfate ions have the same volume of distribution and mean transit time within the lung as blue dextran, a compound unlikely to leave the intravascular space.

Modulation of Airway Sulfate Ion Absorption by Certain Counter Cations: Ammonium ions accelerated the removal of sulfate ions at doses of 0.01 μmole but not at greater doses (Table 3.2). The $t_{\frac{1}{2}}$ of 0.01 μmole sodium sulfate was 33.0 ± 4.7 min compared to 15.7 ± 2.5 min for 0.01 μmole ammonium sulfate. All heavy metal cations, except manganous ion, enhanced the absorption (Table 3.3).

Lack of Release of LDH and Prostaglandins but Release of Histamine by Certain Sulfate Salts: Histamine was released into lung perfusate following an intratracheal injection of 1 μmole $(NH_4)_2SO_4$ (Fig. 3.5). A total of 11.4 μg of histamine was collected in the perfusate from a single injection. The release of prostaglandins from the lung into the perfusate following the intratracheal injection of 1 μmole of either Na_2SO_4, $(NH_4)_2SO_4$ or NH_4Cl was not observed. The bioassay system has been used in other experiments

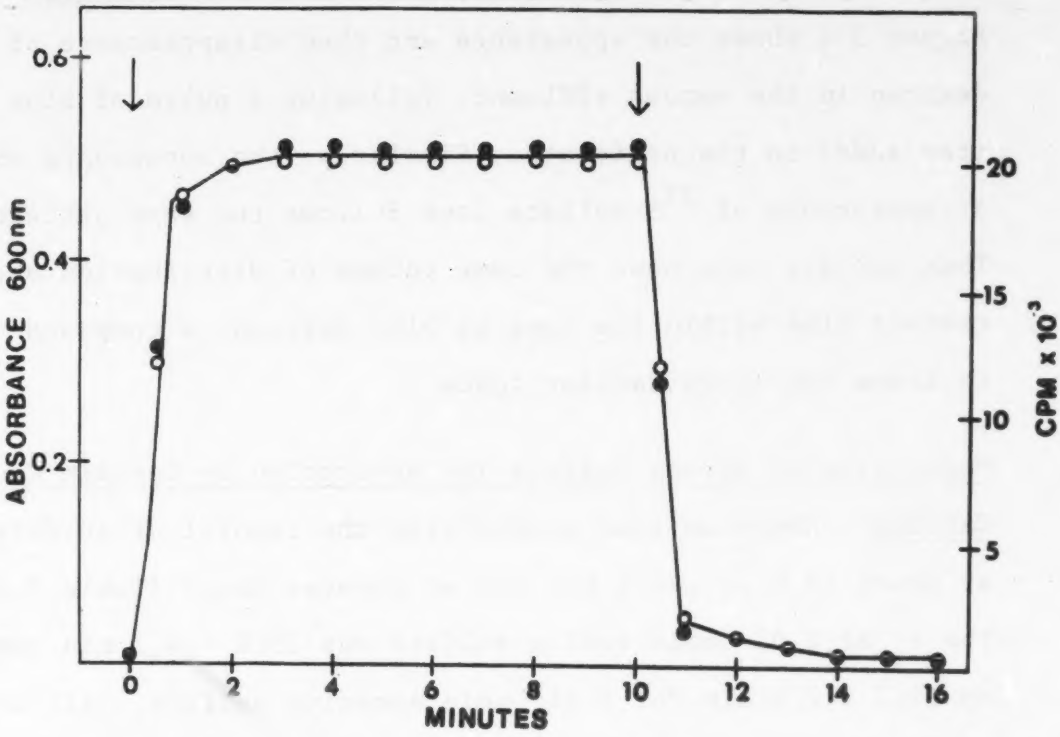


Fig. 3.4. Absence of pulmonary vascular uptake of ^{35}S-sulfate ions. Lungs were perfused continuously with modified Tyrode's medium. A syringe pump provided a pulse of ^{35}S-sodium sulfate and blue dextran in modified Tyrode's solution into the main perfusion line (2 ml/min). The arrows indicate the times at which the syringe pump was turned on (first arrow) and off (second arrow). Blue dextran (o) and ^{35}S-sulfate ions (●) were measured in the pulmonary venous effluent.

TABLE 3.3

Effect of Heavy Metal Counter Cation on the Kinetics of Sulfate Ion Absorption from the Rat IVPL

Compound[a]	Dose per Lung (μmole)	No. of Experiments	Initial k (Minutes^{-1})[b]	$t_{\frac{1}{2}}$ (Minutes)[b]
Na_2SO_4	0.1	11	0.085 ± 0.016	8.12 ± 1.32
$MnCl_2$	0.1	5	0.098 ± 0.013	7.10 ± 1.29[c]
$CoCl_2$	0.1	4	0.169 ± 0.034	4.11 ± 1.00**
$NiCl_2$	0.1	4	0.171 ± 0.028	4.05 ± 0.70**
$ZnCl_2$	0.1	4	0.172 ± 0.018	4.03 ± 0.37**

TABLE 3.3 (Continued)

Compound[a]	Dose per Lung (μmole)	No. of Experiments	Initial k (Minutes^{-1})[b]	$t_{½}$ (Minutes)[b]
$CdCl_2$	0.1	4	0.195 ± 0.047	3.56 ± 0.82**
$FeCl_3$	0.1	4	0.194 ± 0.033	3.56 ± 0.57**
$HgCl_2$	0.1	4	0.224 ± 0.047	3.10 ± 0.62**

[a] All chloride salts were administered intratracheally in 100 μliters of isotonic sucrose, pH 7.4, in the presence of 0.1 μmole $^{35}S\text{-}Na_2SO_4$ (specific activity - 0.65 μCi/mmole). Control was 0.1 μmole $^{35}S\text{-}Na_2SO_4$ alone.

[b] Values are expressed as mean ± s.e.

[c] Paired "t" test between indicated means and mean of control shows no significant difference.

** Paired "t" test between indicated means and mean of control show significant difference $p < 0.05$.

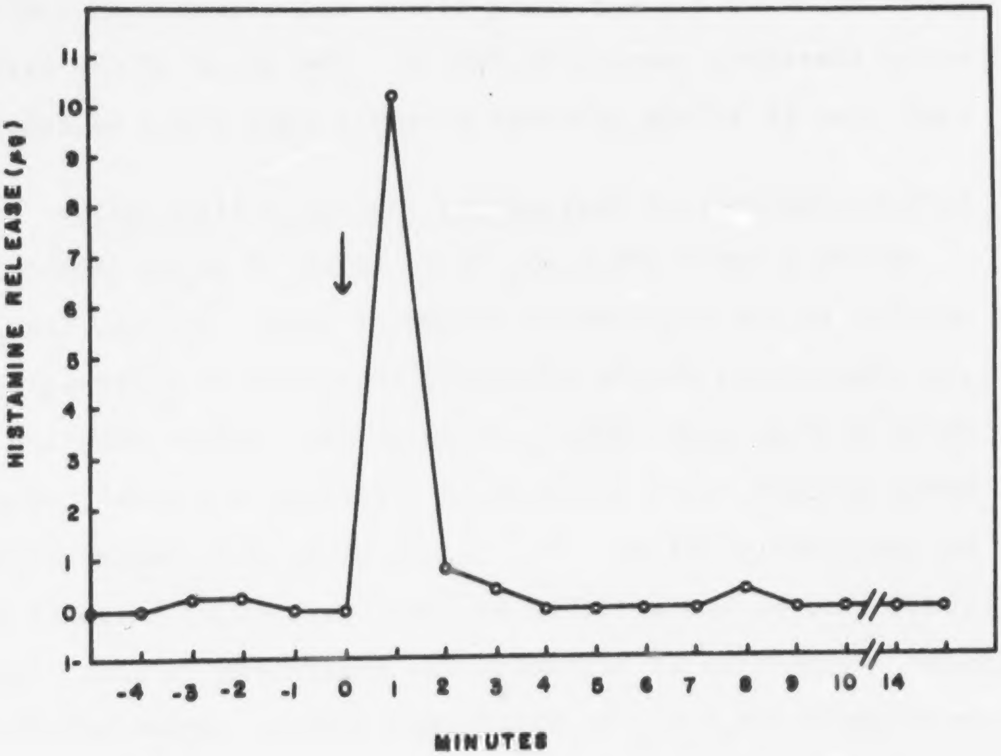


Fig. 3.5. Release of histamine into the lung effluent after intratracheal injection of 1 µmole ammonium sulfate. Injection is indicated by arrow.

to detect the release of about 0.5 ng/ml prostaglandin E_2 and 5 ng/ml prostaglandin $F_{2\alpha}$ from the rat lung. No detectable amounts of LDH were released by intratracheal administration by any of the salts tested. Total LDH level in the rat lung was found to be 63.8 ± 9.9 IU/mg tissue wet weight. An average lung would therefore contain 84,000 IU. The limit of the assay was such that 29 IU/min released from the lung could be detected.

Bronchoconstriction Mediated by Certain Sulfate Salts: A dose of either 1 μmole $(NH_4)_2SO_4$ or 2 μmoles of NH_4Cl caused a reduction in the respiratory volume of lung. Typical tracings of the respiratory volume following injection of these salts are shown in Fig. 3.6. Instillation of the control solution, isotonic sucrose, or 1 μmole Na_2SO_4 produced a minimal reduction in respiratory volume. The reduction in respiratory volume could also be accomplished by the injection of 14 μg of histamine. This dose of histamine was equivalent to that released by 1 μmole $(NH_4)_2SO_4$ in prior experiments. Ammonium chloride was less effective, producing a 26% decrease compared to a 56% for both $(NH_4)_2SO_4$ and histamine. Prior perfusion of the lung with 1.5×10^{-5} M mepyramine maleate added to the Tyrode's medium blocked the reduction of the respiratory volume by both histamine and $(NH_4)_2SO_4$.

Absorption of ^3H_2O by the Rat IVPL: Since histamine was released by ammonium salts, the enhanced sulfate absorption from

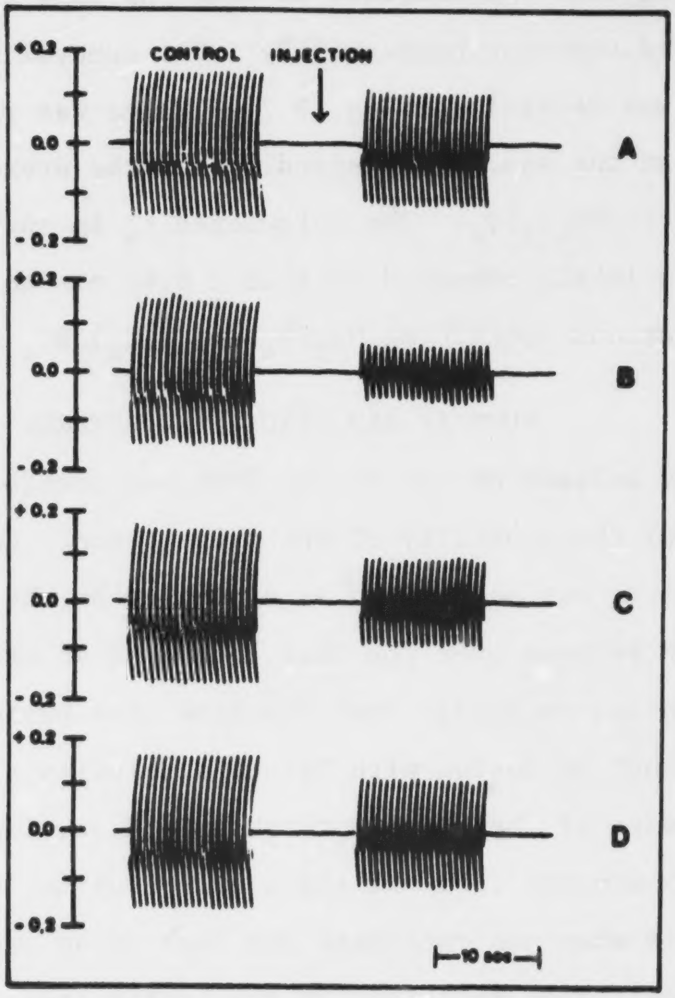


Fig. 3.6. Effects of intratracheal injection of 1 μmole of salts in 0.1 ml isotonic sucrose solution on respiratory volume in the isolated rat lung. A. Isotonic sucrose alone or with sodium sulfate; B. Ammonium sulfate or 14 μg histamine; C. 2 μmole ammonium chloride; and D. Perfusion of lung with 10^{-5} M mepyramine maleate prior to injection of ammonium sulfate, ammonium chloride or 14 μg histamine.

the airways in the presence of ammonium ions could be due to increased vascular permeability. The removal of ^3H_2O from the airway was determined (Fig. 3.7). Water was removed from the airway at the same rate regardless of the presence of 0.1 μmole Na_2SO_4 or $(NH_4)_2SO_4$. The calculated $t_{\frac{1}{2}}$ in sucrose alone was 0.94 ± 0.18 min compared to 1.13 ± 0.42 min and 0.92 ± 0.15 min for solutions containing Na_2SO_4 and $(NH_4)_2SO_4$ respectively.

SUMMARY AND CONCLUDING REMARKS

The release of LDH by the IVPL was monitored as an indication of the viability of the preparation. Cellular integrity of the lung was maintained as evidenced by the lack of significant LDH release over the time course of an experiment. Gross edema failed to appear over the same time period. It was observed that perfusion with Tyrode's solution (25°C, pH 7.4) in the absence of the PVP led to the rapid development of edema within 5 minutes from the start of perfusion. This is understandable when one considers the lack of an oncotic pressure in the perfusion solution, in the latter case.

In the present studies, the removal of sulfate ions from the airway appears to be predominantly by simple diffusion. At very low doses ammonium ion increases the removal process. The $t_{\frac{1}{2}}$ at doses of 0.05 μmole or greater of Na_2SO_4 was 8.4 ± 1.8 minutes.

The heavy metal cations tested, increased sulfate ion absorption from the rat IVPL 199-264% as compared to absorption

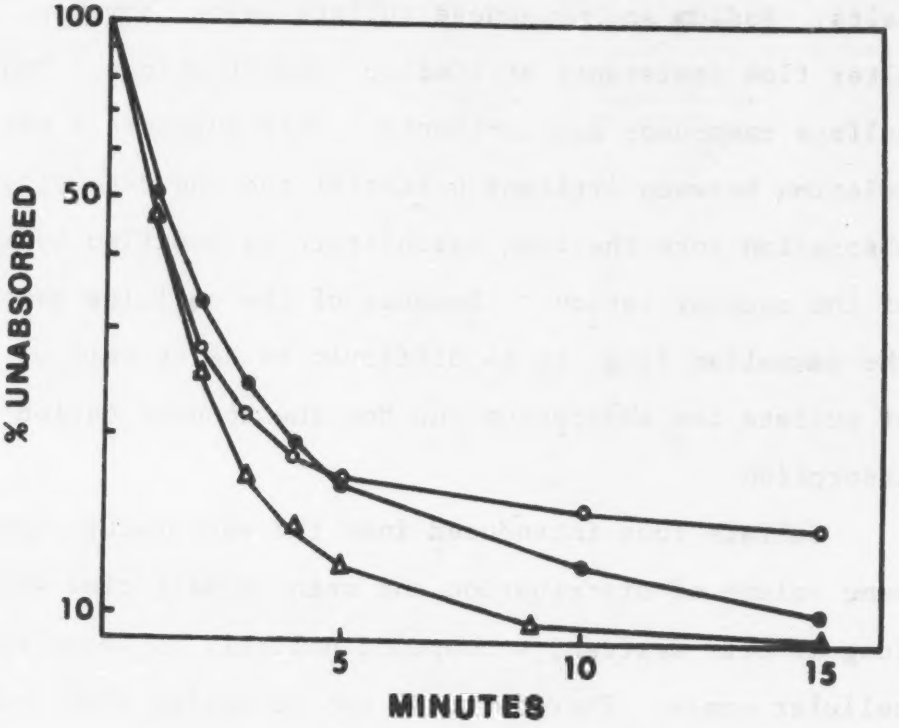


Fig. 3.7. Absorption of ^3H_2O in the isolated, ventilated and perfused rat lung. Open circles, ^3H_2O alone; solid circles, ^3H_2O and 0.10 μmole sodium sulfate; and open triangles ^3H_2O and 0.10 μmole ammonium sulfate.

in the presence of sodium ions. An exception to the above was the case of manganese ion in which sulfate ion absorption did not differ significantly from control.

Amdur (5) and Amdur and Underhill (9) have previously reported an irritant potential associated with certain sulfate salts. Sodium and manganese sulfate salts, however, failed to alter flow resistance at similar concentrations. Thus not all sulfate compounds are irritants. This suggests a possible correlation between irritant potential and the rate of sulfate ion absorption into the lung vasculature as modified by the presence of the counter cation. Because of the cellular complexity of the mammalian lung, it is difficult to be certain of the mechanism of sulfate ion absorption and how the counter cation enhances absorption.

Sulfate ions introduced into the vasculature have the same volume of distribution and mean transit time within the lung as blue dextran, a compound unlikely to leave the intracellular space. Therefore, it can be stated with some certainty that sulfate ion absorption in the rat IVPL is unidirectional.

As in the studies with guinea pig lung fragments, ammonium ions play an important role in the release of histamine in perfused lung. The release of 11.4 μg of histamine by a single dose of 1 μmole of $(NH_4)_2SO_4$ represented an almost complete degranulation (see Shore et.al. (66) for histamine content of tissues). All other cations studied, failed to release detectable amounts of histamine. No measurable amounts of LDH were released by any salt tested. Although prostaglandins were specifically sought for, none were released. Using the same preparation for other studies, prostaglandins are detected in the perfusate.

Intratracheal instillation of ammonium sulfate solutions decreased the respiratory volume of the preparation by 56 percent while ammonium chloride caused only a 26 percent decrease. Histamine (14 μg) applied intratracheally also decreased the respiratory volume by the same percentage. The release of histamine was shown to be rapid and followed the same time course as the decrease in respiratory volume. Histamine appeared to be the only vasoactive hormone elaborated. Since prior perfusion with an H-1 antihistamine prevented a decrease in the respiratory volume by ammonium sulfate, histamine is most likely the only mediator.

Little or no changes in the vascular permeability could be observed with doses of $(NH_4)_2SO_4$ causing histamine liberation. The $t_{\frac{1}{2}}$ for the removal of ^3H_2O from the rat IVPL of 1.01 ± 0.14 minutes compared favorably with that reported by Taylor et.al. (74) for deuterium oxide of 0.80 minutes. The increased rate of removal of sulfate ions in the presence of ammonium ions is unlikely to be due to changes in vascular permeability mediated by histamine.

SECTION 6

SULFATE ION ABSORPTION IN THE RAT LUNG IN VIVO

INTRODUCTION

The respiratory tract epithelium behaves as a highly porous membrane permeable to a number of solutes. Removal of organic compounds from the airway has been studied by Schanker and his colleagues. Schanker and coworkers administered intratracheally a small volume (0.1 ml) of Krebs-Ringer phosphate solution containing ^{14}C-labelled compounds to anesthetized rats. Removal was determined by assay of the ^{14}C-radioactivity remaining in the lung. A number of lipid insoluble neutral compounds, including urea, erythritol, mannitol and sucrose, administered over a 100-1,000 fold range of concentration, disappeared from the lungs at rates directly proportional to the concentration (22). The relative rates of absorption ranked in the same order as the diffusion coefficients of the compounds. Simple diffusion appears to account for removal. Enna and Schanker suggested that, due to the extremely low lipid solubility of these compounds, absorption was predominantly by passage through aqueous channels or pores in the membrane rather than through lipid regions. Diffusion through at least three different populations of pore size could explain the absorption of these compounds. The smallest diameter pore prevents the diffusion of all the saccharides relative to that of urea. A larger diameter pore allows the diffusion of only erythritol, while the largest pore size permits the passage of all compounds except dextran (MW - 70,000).

In another study involving organic anions and cations, sulfanilic acid, tetraethyl ammonium ion, p-aminohippuric acid, and p-acetylaminohippuric acid, and procaineamide ethobromide appeared to be absorbed by diffusion through aqueous pores since their absorption was non-saturable and roughly related to molecular size rather than to partition coefficient (23).

Phenol red, a lipid insoluble organic anion is an exception to the above cases. This compound is absorbed from the rat lung not only by diffusion but also in part by a carrier-type transport process ($t_{\frac{1}{2}}$ = 20 minutes). This process, which becomes saturated at high concentrations of the dye, is inhibited by certain organic anions including benzylpenicillin and cephalothin (24). The main barrier to the diffusion of water soluble compounds is the alveolar membrane. In studies with the isolated perfused dog lung, Taylor and Gaar (73) calculated an equivalent pore radius of 8-10 Å for the alveolar membrane and a much larger radius for the capillary endothelium.

Lipid soluble compounds are thought to be absorbed mainly by diffusing through lipoid regions of the membrane. Burton and Schanker (13) administered five antibiotics intratracheally to anesthetized rats. The $t_{\frac{1}{2}}$ ranged from 1.9 to 33 minutes. Chloramphenicol was absorbed most rapidly followed by doxycycline, erythromycin and tetracycline, with benzyl penicillin showing the slowest rate. A comparison of pulmonary absorption rate, molecular weight and chloroform/water partition coefficient of the drugs indicated that lipid solubility was more closely associated with the relative rate of absorption than molecular size. Corticosteroids are rapidly absorbed from the respiratory tract with $t_{\frac{1}{2}}$ of absorption ranging from 1.0-1.7 minutes (14).

In other studies on lipid soluble compounds Normand _et.al_. (56) investigated the permeability of alveoli and capillaries in the fluid filled lungs of the fetal lamb. They reported that urea, thiourea and N-ethylthiourea penetrated the alveolar wall at rates which increased with the lipid solubility of the compounds. Earlier, Taylor _et.al_. (74) in a study of the alveolar membrane of the isolated perfused dog lung, reported that the permeability coefficient of a lipid soluble compound, dinitrophenol, was much greater than that of lipid insoluble compounds, such as glucose and urea.

Only a few studies of the movement of simple anions across the lung have appeared. Gatzy (32) proposed that chloride ion transport accounts for the potential difference found across the alveolar membranes of the amphibian lung. Chloride ion transport also is found in other epithelia such as the toad bladder (27) and frog epithelium (39). Using the permeability coefficient of sodium ion, the $t_{\frac{1}{2}}$ for sulfate ion transport in the turtle lung can be calculated as 27.8 minutes (19). Sulfate ion transport in mammalian lungs has not been reported previously.

It must be pointed out that in all studies cited, the actual site of solute absorption is unknown. Absorption could be occurring across the alveolar epithelium, across the bronche-olar epithelium of the airways or at a combination of these sites. Burton and Schanker (12) have reported that lung absorption rates are increased by at least two-fold when solutes are administered as aerosols.

The studies presented in this chapter were designed to determine the manner by which the mammalian lung disposes of inspired sulfate ions. Possible modulation of the absorption process by the associated cation was also investigated.

METHODS

In Vivo Rat Lung Preparation: The technique is a modification of the method of Enna and Schanker (23). Female Sprague Dawley Rats weighing 150-200 g were anesthetized with sodium pentobarbital (35 mg/kg). The animal was placed on its back on an animal board and the limbs were secured with masking tape. After exposing the trachea through a longitudinal incision in the neck, the trachea was cut transversely halfway through between two tracheal rings. Two centimeters of a 4 cm long polyethylene cannula (PE 240) was inserted into the trachea to a point approximately 0.5 cm above the hylum and sutured. The incision was then covered with sterile gauze dipped in Tyrode's medium (pH 7.4). The mounting board was placed in an upright position. One-tenth milliliter of an isotonic sucrose solution (300 mM, 25°C, pH 7.4) containing ^{35}S-sodium sulfate alone and with variable chloride salts, was injected into the lungs via the tracheal cannula through a 1.5 inch 22 gauge needle attached to a 100 μl syringe (Hamilton). The solution was injected over a 1 to 2 second interval. The needle and syringe were withdrawn completely, the animal removed from the mounting board, laid on its stomach, and maintained under light anesthesia during the specified experimental time period.

Absorption of ^{35}S-Sulfate Ions from the Rat Lung: One-tenth milliliter of the isotonic sucrose solution containing either 1.0, 10.0 or 100.0 nanomole ^{35}S-sodium sulfate (specific activity - 0.014 μCi/nanomole) was injected into the lungs via the tracheal cannula. Absorption of sulfate ions was allowed to occur for various times between 0 and 120 minutes. One minute before the end of the absorption period, removal of the lungs was begun. The pleural cavity was carefully opened and the trachea gently separated from surrounding tissue. At the end of the absorption period, the blood supply to the lungs was quickly stopped by cutting around both sides of the lung. The lungs with the heart, a portion of the trachea and the trachea cannula attached were removed from the body. The heart, thymus gland, and the esophagus were trimmed away and the cannula removed. The lungs and trachea were immediately prepared for the determination of ^{35}S-radioactivity remaining in the lungs by oxygen combustion described previously. These preliminary experiments were used to characterize the mechanism of sulfate ion removal and to establish the approximate half-life.

Modulation of Sulfate Ion Absorption by the Counter Cation: To investigate the absorption of sulfate ions in the presence of other cations, an unlabelled chloride salt of the cation in question was dissolved in the isotonic sucrose solution together with ^{35}S-sodium sulfate (specific activity - 0.014 μCi/nanomole). Injections of 0.1 ml isotonic sucrose solution were used containing 0.1 nanomole ^{35}S-sodium sulfate and either 0.1, 1.0 or 10.0 nanomole of the chloride salt. Absorption was allowed to proceed for 30 minutes, approximately equal to the $t_{\frac{1}{2}}$ of sulfate ion absorption from the rat lung. As before, one minute prior to the end of the absorption period, removal of the lungs was begun and the ^{35}S-radioactivity remaining in the lung determined. Chloride salts studied in this manner were ammonium, cadmium, cobaltous, ferric, manganous, mercuric, nickelous, and zinc.

Modulation of Sulfate Ion Absorption by pH: To investigate the absorption of sulfate ions under varying pH conditions, the isotonic sucrose solution containing $^{35}S-Na_2SO_4$ was adjusted to a known pH (pH 4.4-9.4) with either HCl or NaOH prior to injection. Injections of 0.1 ml of these solutions were used containing 0.1 namomole ^{35}S-sodium sulfate (specific activity - 0.014 μCi/nanomole). Absorption was allowed to proceed for 30 minutes. As before, one minute prior to the end of the absorption period removal of the lungs was begun and ^{35}S-radioactivity remaining in the lung determined.

Exposure of Rats to Nickel Chloride Aerosol Prior to the Determination of Sulfate Ion Absorption: The inhalation exposures were conducted in a plexiglass exposure chamber expecially designed for exposure to particulate aerosols. The animals were isolated in individual cells which allowed only their heads to be exposed to the aerosol. All exposures were for two hours. A fluid atomizer generator (Environmental Research Corporation, Model 7330) was used to generate the nickel chloride aerosol from solutions ranging in concentration from 2.61 to 13.05 gm/l of deionized water. Samples were collected on membrane filters having 0.22 μm porosity for determining the concentration within the chamber. The deposited salt was eluted from the filter by placing it in a flask containing 19.8 ml of deionized water and 0.2 ml of HNO_3. After shaking for several hours the resulting solution was analyzed using a Perkin-Elmer Model 306 atomic absorption spectrophotometer with a Model 2100 heated graphite accessory. For monitoring the aerosol exposure by particle size distribution, a Royco Instrument Model 225 Particle Counter with a Model 507 module was used. The mass median diameter of the $NiCl_2$ aerosol was 1 μm or below in these experiments. The mass median aerodynamics diameter was determined to be less than or equal to 2μm. Control animals were exposed for 2 hours to a deionized water aerosol.

After the exposure period, the animals were removed from their cells and 4 animals were used in the determination of the amount of Ni^{++} deposited after a 2 hour exposure. Their lungs were excised, ashed, and analyzed as before for Ni^{++} content.

The exposed animals were used to determine sulfate ion absorption. A tracheal cannula was inserted as before. Injections of 0.1 ml isotonic sucrose containing 0.1 nanomole ^{35}S-sodium sulfate (specific activity - 0.014 μCi/nanomole) pH 7.40. Absorption was allowed to proceed for 30 minutes. The lungs were removed at the end of this time and ^{35}S-radioactivity determined.

Materials: ^{35}S-sodium sulfate was purchased from New England Nuclear.

RESULTS

Kinetics of Sulfate Ion Removal from the Airways: Fig. 4.1 shows a semilogarithmic plot of the removal of sodium ^{35}S-sulfate

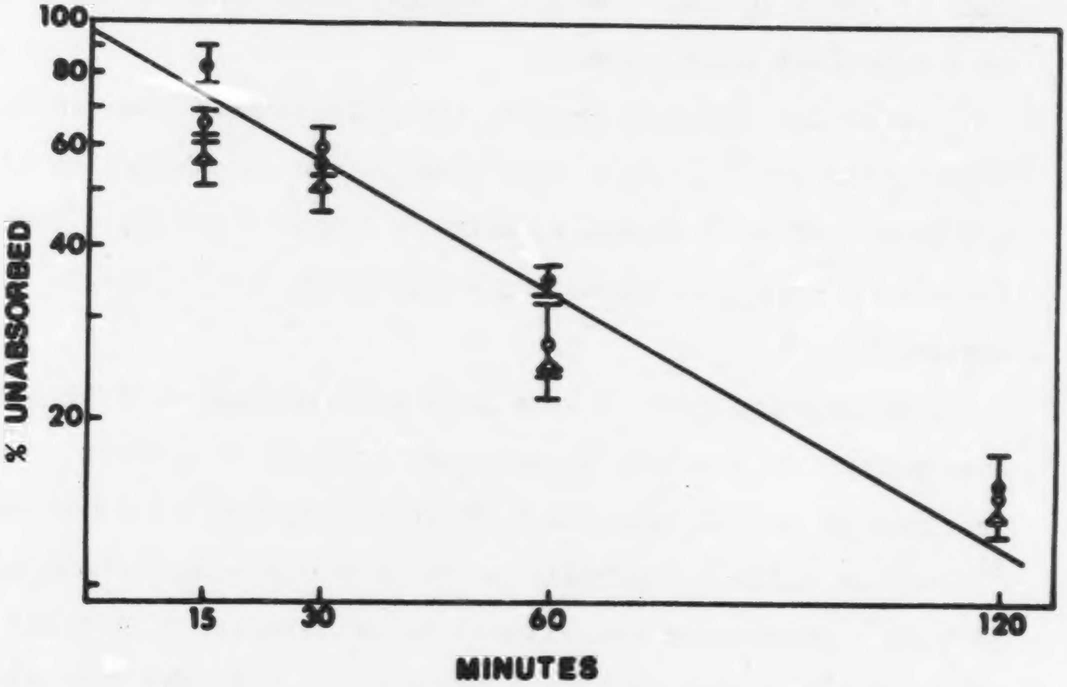


Fig. 4.1 Percent sulfate ion unabsorbed by the rat lung versus time in the presence of various concentrations of sodium sulfate. Each point is the mean s.e. of three animals. Closed circles, 1.0 nanomole; open circles, 10.0 nanomole; open triangles, 100 nanomole.

from the lung following intratracheal administration. Three concentrations, 1.0, 10.0 and 100.0 namomole Na_2SO_4 were used. Although the concentration of Na_2SO_4 varied over a 100-fold range, the percentage absorbed at a given time was constant. The total amount of Na_2SO_4 absorbed was proportional to the initial concentration administered (Table 4.1). These results suggested that absorption occurred by a nonsaturable process, such as simple diffusion. The half-life for sulfate absorption in the presence of sodium ions, calculated from the slopes of these lines was found to be 34.5 minutes.

Modulation of Airway Sulfate Ion Absorption by Certain Counter Cations: The removal of sulfate ions was accelerated in the presence of certain cations (Table 4.2). The dose of cation at which maximum augmentation of sulfate absorption occurred was variable. Co^{++} and Hg^{++} ions produced maximal effect at 0.1 nanomole. Cd^{++} and Ni^{++} were of intermediary potency. Fe^{+++}, Zn^{++} and NH_4^+ were the least effective reaching maximal effect at 10.0 nanomoles. As was observed in the case of the isolated perfused rat lung, Mn^{++} failed to alter sulfate ion absorption. Sodium chloride depressed slightly the sulfate ion absorption when present at 10 nanomole.

Modulation of Airway Sulfate Ion Absorption by pH: The absorption of sulfate ions from the rat lung in vivo was enhanced at pH values departing from physiological values (Fig. 4.2).

TABLE 4.1

Absorption of ^{35}S-Sodium Sulfate from the Rat Lung

Concentration of Na_2SO_4 (nanomole)	No. of Animals	30 Min. Absorption % Dose ± s.e.	Sulfate Ion Amount (μg)
1.0	11	43.2 ± 2.5[a]	0.041 ± 0.002
10.0	3	42.0 ± 5.0[a]	0.403 ± 0.048
100.0	3	47.4 ± 6.8[a]	4.550 ± 0.653

[a] No statistical difference at $p < 0.01$.

TABLE 4.2

Effect of Counter Cation on Pulmonary Absorption of ^{35}S-Sulfate Ions

Salt[a]	Concentration (nanomole)	No. of Animals	30 Minute Absorption Sulfate Ion		% Enhancement over Control
			% Dose ± s.e.	Amount (ng)	
Na_2SO_4 alone	1.0	11	43.2 ± 2.4	41.5 ± 2.4	0.0
$MnCl_2$	1.0	8	44.8 ± 1.7	43.0 ± 1.6	3.7 ± 3.9[b]
NH_4Cl	10.0	7	54.1 ± 2.3	51.9 ± 2.2	25.2 ± 5.3
$ZnCl_2$	0.1	5	49.3 ± 5.2	47.0 ± 5.0	14.2 ± 12.0
	1.0	5	55.2 ± 3.1	53.0 ± 3.0	27.8 ± 7.2
	10.0	5	64.2 ± 2.8	61.6 ± 2.7	48.6 ± 6.5
$FeCl_3$	0.1	6	50.3 ± 4.9	48.0 ± 5.0	16.4 ± 11.3
	1.0	6	53.8 ± 1.7	51.7 ± 1.6	24.5 ± 3.9
	10.0	5	72.4 ± 2.9	69.5 ± 2.7	46.2 ± 6.7
$CdCl_2$	0.1	6	52.4 ± 3.0	50.3 ± 3.1	21.3 ± 6.9
	1.0	8	57.2 ± 4.3	54.9 ± 4.1	32.4 ± 9.9
	10.0	7	56.6 ± 2.6	54.3 ± 2.4	31.0 ± 6.0

TABLE 4.2 (Continued)

Salt[a]	Concentration (nanomole)	No. of Animals	30 Minute Absorption Sulfate Ion		% Enhancement over Control
			% Dose ± s.e.	Amount (ng)	
$NiCl_2$	0.1	4	54.8 ± 2.2	53.2 ± 2.1	26.7 ± 5.0
	1.0	10	56.8 ± 1.7	54.5 ± 1.6	31.5 ± 3.9
	10.0	5	56.5 ± 1.3	54.2 ± 1.2	31.0 ± 3.0
$HgCl_3$	0.1	5	55.0 ± 2.1	53.3 ± 2.0	27.3 ± 4.9
	1.0	6	57.2 ± 4.6	54.9 ± 4.6	32.4 ±11.1
	10.0	5	55.3 ± 3.5	53.1 ± 3.4	28.0 ± 7.8
$CoCl_2$	0.1	8	62.2 ± 2.6	60.0 ± 2.2	44.0 ± 6.0
	1.0	10	56.8 ± 2.8	54.5 ± 2.5	31.5 ± 6.0
	10.0	8	56.9 ± 2.8	54.6 ± 2.6	31.7 ± 6.0
NaCl	10.0	11	36.6 ± 3.0	35.1 ± 2.8	-15.3 ± 6.9

[a] All chloride salts were studied in the presence of 1.0 nanomole $^{35}S\text{-}Na_2SO_4$ (specific activity - 0.014 μCi/mmole).

[b] No statistical difference over control at $p < .001$.

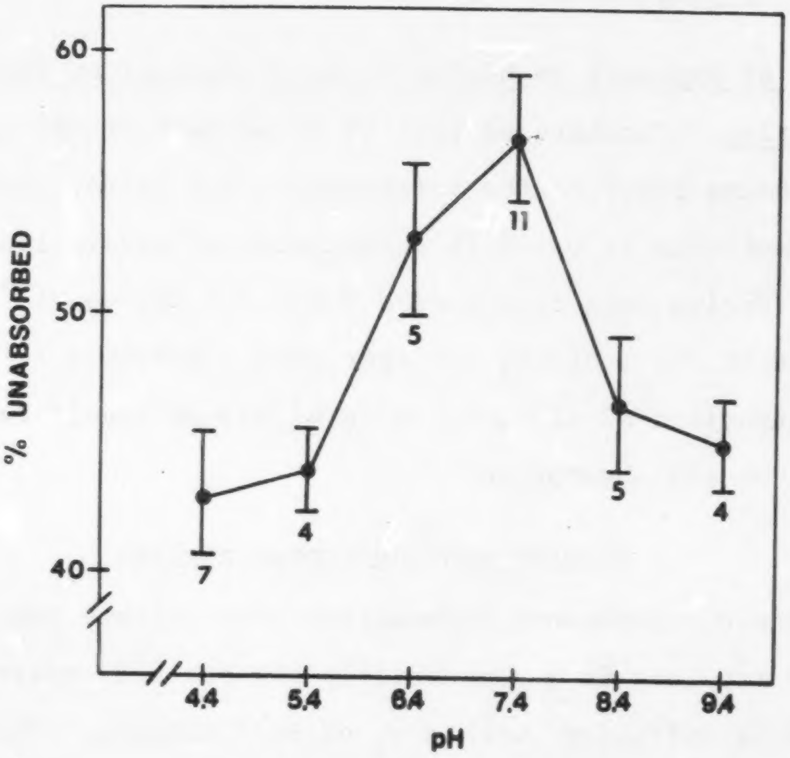


Fig. 4.2. Percent sulfate ion unabsorbed by the rat lung after 30 minutes under varying pH conditions in the presence of 1.0 nanomole ^{35}S-sodium sulfate (specific activity - 0.014 µCi/mmole). Each point represents the mean ± s.e. The number of animals used at each pH is given.

In basic solutions the maximum enhancement over control values (pH 7.4) of 26.9 ± 4.4% were observed at pH 9.4. In acid solutions, a maximum enhancement of 32.4 ± 5.6% was observed at pH 4.4. Sulfate ion absorption was relatively constant between pH 6.4 and 7.4.

Effect of Exposure to Nickel Chloride Aerosol on Sulfate Ion Absorption: Exposure of rats to an aerosol of 480 $\mu g/m^3$ $NiCl_2$ for 2 hours prior to the determination of sulfate ion absorption led to a 12.0 ± 2.7% enhancement of absorption (Table 4.3). During this time period 0.856 ± 0.060 μg Ni^{++} was deposited in the lungs of the test rats. Exposure to aerosol concentrations of 113 and 279 $\mu g/m^3$ had no significant effect on the absorption.

SUMMARY AND CONCLUDING REMARKS

These experiments demonstrate that sulfate ion removal by the rat lung is a nonsaturable process and appears to occur by simple diffusion, with a $t_{\frac{1}{2}}$ of 34.5 minutes. This $t_{\frac{1}{2}}$ compares favorably with the value of 27.8 minutes for sulfate ion removal in the turtle lung reported by Deitchman and Paganelli (19). We have also observed that ammonium ions and certain heavy metals enhance the absorption whereas sodium chloride depresses slightly the absorption of sulfate.

As was shown in the isolated perfused rat lung Mn^{++} failed to effect sulfate ion absorption. The ability of

TABLE 4.3

Effect of 2 Hour Exposure to Nickel Chloride Aerosol Prior to Determination of Pulmonary Absorption of ^{35}S-Sulfate Ions

Concentration of $NiCl_2$ Aerosol (μg Ni/m^3)	No. of Animals	Amount of Ni^{++} Deposited (μg/lung)	30 Min. Absorption Sulfate Ions[a]		% Enhancement over Control
			% Dose ± s.e.	Amount (μg)	
Control	5	0	57.9 ± 2.0	0.056 ± 0.002	0
113	6	0.141 ± 0.046	60.0 ± 1.7	0.058 ± 0.002	4.6 ± 2.9
279	5	0.189 ± 0.039	60.8 ± 2.4	0.058 ± 0.002	5.0 ± 4.2
480	6	0.856 ± 0.060	64.9 ± 1.6**	0.062 ± 0.022**	12.0 ± 2.7**

[a] In the presence of 1 nanomole ^{35}S-sodium sulfate (specific activity - 0.014 μCi/nanomole).

** Significant difference from control animals $p < .05$.

heavy metals, given as an aerosol to test animals, was also demonstrated to enhance the absorption of sulfate ions. Sulfate ion absorption was enhanced under basic and acidic conditions, but remained relatively constant between pH 6.4 to 7.4.

Assuming the sulfate aerosol levels reported in the CHESS study (26) a total burden of 0.12 μg per hour would be inhaled by the rat exposed to ambient levels of particulate sulfates of 20 μg per cubic meter. This calculation assumes that all of the suspended sulfate would be deposited in lung and that the rat would have a tidal volume of 1 cc and a respiratory rate of 100 breaths per minute. The rate of absorption was the same over a 100 fold range of 0.096 μg of sulfate to 9.6 μg of sulfate ion given in this study. From the data presented, 31 percent of 0.04 μg of the inhaled sulfate from the one hour burden would remain after one hour post exposure. If one were to assume the same rate of removal from the human lung as for the rat lung, then 7.20 μg of sulfate would be inhaled and 2.23 μg would remain after 1 hour of exposure.

It must be pointed out that in all studies cited, including the present one, the actual site of solute removal by the lung is unknown. Burton and Schanker (12) have reported that lung absorption rates are increased by at least two fold when solutes are administered as aerosols. While sulfate ions are readily transported from the lumen of the lung, residual sulfate concentrations are likely to remain. Histamine release and bronchoconstriction may as a consequence occur.

SECTION 7

DISCUSSION

Until recently the prime source of sulfate air pollution was the atmospheric oxidation of sulfur dioxide (SO_2). Measurements of ambient sulfate levels in the Hudson River Valley during 1970-1971 indicate that ammonium sulfate is a principal component of sulfate residues in the atmosphere (Dr. R. Bradow, personal communication). The automobile catalytic converter has introduced a new source of sulfate in the form of sulfuric acid mist. Ammonia and other cations present in the environment probably convert this mist to a mixture of sulfate salts and sulfuric acid.

Amdur (5) and Amdur and Corn (6) measured the increase in pulmonary resistance following the inhalation of zinc ammonium sulfate, zinc sulfate and ammonium sulfate aerosols. Although ammonium sulfate was the least potent salt, it was many times more irritating than its parent compound, SO_2. In further studies, Amdur and Underhill (9) demonstrated that an equivalent amount of sulfur present as SO_2 gas produced a lesser irritant response than sulfuric acid and most sulfate salts. Exceptions to this were ferrous sulfate and manganous sulfate. Thus, not all sulfate salts are irritant in nature.

The observations of McJilton et.al. (52) support the concept that certain sulfate salts are bronchoconstrictors. Nadel et.al. (53) have shown that the inhalation of a zinc ammonium sulfate aerosol increases pulmonary resistance in guinea pigs as does inhalation of a histamine aerosol.

We have been able to demonstrate that unsensitized guinea pig lung fragments (ULF) incubated with a variety of ammonium salts release significant quantities of histamine. The most efficacious, ammonium sulfate (100 mM), shows maximal histamine release after 30 minutes. The ammonium sulfate mediated release is equal to 97% of the total histamine stores. Cell lysis through osmotic shock is unlikely since equal concentrations of sodium chloride fails to release histamine. Lysis of the mast cells is not likely since neither LDH nor DNA are released into the supernatant in the presence of ammonium sulfate. Total DNA in the fragments remains constant in the presence of ammonium sulfate. Total DNA in the fragments remains constant in the presence of concentrations of ammonium sulfate known to release histamine. Equal concentrations of sodium sulfate also fail to release histamine, supporting the concept that only certain sulfate salts have biological actions. These studies suggest that the inhalation irritation associated with certain sulfate salts may be a function of their ability to release histamine in the presence of ammonium ion.

The intracellular sulfate ion space in both ULF and SLF decreases in the presence of $(NH_4)_2SO_4$ when compared to that measured in the presence of Na_2SO_4. Since histamine release only occurs in the presence of $(NH_4)_2SO_4$, the decrease in the intracellular sulfate ion space is probably associated with the histamine release process.

Sensitized guinea pig lung fragments (SLF) have been shown to release histamine and slow reacting substance of anaphylaxis (SRS-A) on stimulation by the immunoglobulin E (IgE) mediated antibody-antigen reaction (71). This process has been shown to be modulated by the cAMP and cGMP systems (44,57,58,65). Ammonium sulfate mediated histamine release from ULF cannot be modulated by drugs acting on these systems.

Sulfate ion uptake by ULF and SLF is uneffected by pharmacological agents known to modulate cellular cAMP and cGMP levels. The absorption of sulfate ions does not appear to be highly dependent on the availability of metabolic sources of energy. At high concentrations of potent metabolic inhibitors only partial inhibition of sulfate ion uptake is observed. Phloretin has been reported to inhibit chloride and sulfate uptake by human red blood cells (83), however, phloretin has no effect on the sulfate ion uptake by ULF and SLF.

Data presented here, concurs with the observations of others (46), that the metachromasia associated with Acridine Orange binding to heparin is a function of ionic strength.

The number of Acridine Orange binding sites found in our experiments of 3.53 ± 0.10 binding sites per disaccharide unit corresponds favorably with that reported by Lagunoff (46) of 3.31 ± 0.09 binding sites per dissacharide unit. The total number remains constant with increasing ionic strength. Stone and Bradley (72) and Lagunoff (47) suggest that the binding sites for Acridine Orange and histamine on the heparin macromolecule are identical. Since we observe a decrease in the extent of Acridine Orange binding to the heparin macromolecule with increasing ionic strength, a local increase in the ionic strength within the granule is likely to cause displacement of histamine bound to heparin. Since the mast cell granule is freely permeable to the external ionic environment, intracellular uptake of ammonium or sulfate ions could result in the displacement of bound histamine.

In experiments with the isolated, perfused and ventilated lung (IVPL), the removal of sulfate ions from the airway appears to be predominantly by simple diffusion. Absorption of sulfate ions in the reverse direction, specifically from the vasculature into the lung, could not be demonstrated. At very low doses ammonium ion increases the removal process. The $t_{\frac{1}{2}}$ at doses of 0.05 μmole or greater was 8.4 ± 1.8 minutes. The heavy metal cations tested significantly enhanced the absorption of sulfate ions from the airways. An exception to this rule was manganous ion, which had no effect over control.

Intratracheal instillation of ammonium sulfate solutions decreases the tidal volume of the preparation by 56% while ammonium chloride causes only a 26% decrease. Histamine (14 μg) applied intratracheally also decreases the tidal volume by the same percentage. As in the studies with guinea pig slices, ammonium ions played an important role in the release of histamine in perfused lungs. Histamine appears rapidly in the perfusate in about the same amount, 11 μg, and produces an equivalent decrease in tidal volume. The release of histamine is rapid and follows the same time course as the decrease in tidal volume and represents an almost complete degranulation. Although prostaglandins were specifically sought for, none were released. Histamine appears to be the only vasoactive hormone elaborated. Since prior perfusion with an H-1 antihistamine prevents a decrease in the tidal volume by ammonium sulfate, histamine is most likely the principle mediator. All the other cations tested failed to release histamine or LDH into the lung effluent.

In the final set of studies it is shown that sulfate ion removal by the rat lung in vivo is a non-saturable process and appears to occur by simple diffusion. A $t_{\frac{1}{2}}$ of 34.5 minutes is observed. Deviations from the physiological pH and the presence of certain cations lead to an enhanced absorption of sulfate ions from the airways. As in the case of the rat IVPL, manganous ions failed to modulate absorption.

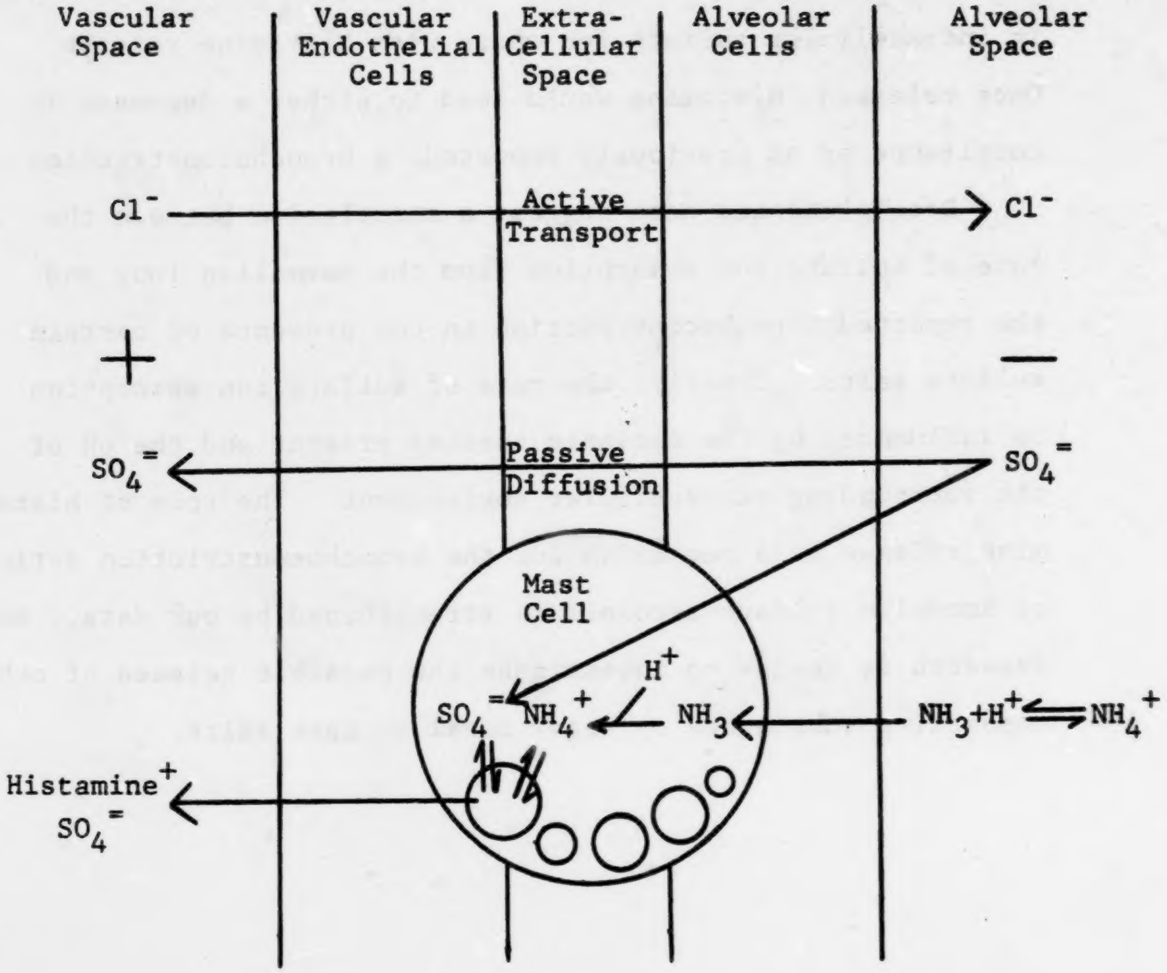
The more rapid diffusional process found in IVPL was not detected. Ammonium ions augment the release of histamine in lung fragments and the removal of sulfate ions in both perfused and living lungs. In the intact animal ammonia is always present in the blood (0.1 mM) and the level of intracellular ammonium ions within the lung may be great enough to obscure the more rapid process seen in IVPL. Other factors such as limitations on the redistribution and elimination of sulfate ions from the blood or feed-back effects from the release of histamine or other vasoactive substances could also alter the removal of sulfate ions from the lung in vivo. As a consequence, one should consider the perfused lung model as the simplest case. Until the intraluminal concentration of sulfate compounds is determined from the inhalation of ambient levels of particulate sulfate compounds, it is difficult to predict which process will predominate.

In all systems tested there is a positive correlation between the irritant potential associated with a specific sulfate salt (5,9), and the rate at which sulfate ions are cleared from the lung.

Althouth most heavy metals are known to exert biological effects through combination with sulfhydryl groups, they also combine with hydroxyl, carboxyl, imidazole and amine groups (59,61). These interactions can lead to membrane changes. The enhanced sulfate ion absorption observed in the experiments presented is probably related to membrane changes resulting from these interactions. The transport of ions across absorbing or excreting membranes has been shown to be sensitive to the action of heavy metal ions (11,28,35,45,75).

Based on these observations, we wish to suggest a possible mechanism for the absorption of sulfate ions and observed histamine release due to ammonium and sulfate ions. The proposed model is depicted in Figure 5.1. Gatzy (33) has demonstrated that chloride ions are actively transported from the vasculature into the lungs. This results in an electrochemical gradient, with the airways being negative with respect to the capillary lumen (32). This electrochemical gradient favors the diffusion of sulfate ions from the airways to the vascular space. Absorption of sulfate ions from the vasculature into the lungs fails to occur, since it is against the electrochemical gradient. Sulfate ions can also diffuse into mast cells. Ammonium ions can dissociate in solution ($pK_a = 9.24$) to hydrogen ion and ammonia. Ammonia is lipid soluble and can diffuse freely across the cell membrane because it is uncharged. Once in the cell ammonium ion can be formed.

The mast cell granule is freely permeable to the cellular ionic environment. Ammonium and sulfate ions could diffuse into the granule and result in the ion exchange reaction depicted in Figure 5.1, leading to the release of $(\text{histamine})_2SO_4$.


Vascular Space
Vascular Endothelial Cells
Extra-Cellular Space
Alveolar Cells
Alveolar Space
Cl⁻
Active Transport
Cl⁻
+
—
SO₄⁼
Passive Diffusion
SO₄⁼
Mast Cell
H⁺
SO₄⁼ NH₄⁺
NH₃
NH₃+H⁺ ⇌ NH₄⁺
Histamine⁺
SO₄⁼

Within the granule:

$$2(\mathrm{HistNH_3}^{+-}\mathrm{SO_4\text{-}Hep\text{-}P}) + 2\mathrm{NH_4}^{+} + \mathrm{SO_4}^{=} \rightleftarrows 2(\mathrm{NH_4}^{+-}\mathrm{SO_4\text{-}Hep\text{-}P}) + (\mathrm{HistNH_3}^{+})_2{}^{=}\mathrm{SO_4}$$

Figure 5.1. Proposed mechanism for sulfate ion absorption and release of histamine in the presence of ammonium sulfate.

Such a model would be consistent with the concomitant decrease in intracellular sulfate ion space with histamine release. Once released, histamine would lead to either a decrease in compliance or as previously reported, a bronchoconstriction.

Date presented here suggest a correlation between the rate of sulfate ion absorption from the mammalian lung and the reported bronchoconstriction in the presence of certain sulfate salts. Clearly, the rate of sulfate ion absorption is influenced by the cationic species present and the pH of the surrounding extracellular environment. The role of histamine release as a mechanism for the bronchoconstriction action of ammonium sulfate aerosols is strengthened by our data. More research is needed to investigate the possible release of other vasoactive substances by heavy metal sulfate salts.

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